THE OESTROUS CYCLE IN THE RAT

AND ITS

ASSOCIATED PHENOMENA

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ASSOCIATED PHENOMENA

BY

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MEMOIRS OF THE UNIVERSITY OF CALIFORNIA

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I. INTRODUCTION

It has long been known that sexual reproduction in the Mammalia takes place when the female is in a particular physiological condition known as oestrus. It has also been known since the time of Pouchet and Bischoff that in the absence of copulation ovulation may nevertheless occur spontaneously at oestrus. In fact the only exceptions to this rule known at the present time would appear to be found in the cat and possibly in the rabbit. Oestrus and consequently ovulation are also known to be cyclic in many forms.

There is no need to explain the profound importance of an infallible method for recognizing what is thus the periodic function of the ovary. The embryologist, moreover, is interested in the precise time of ovulation inasmuch as knowledge of the sequence of events in the earliest development of mammals depends on such information.

While in some animals oestrus is outspoken either by peculiarities of behavior or by easily recognizable signs, in other forms this is not true. To the latter group belong some animals like the rat, of major scientific importance on account of their ready availability for experimental work.

More than one method might be employed in attempts to recognize the oestrous cycle. In lieu of various signs of oestrus, the chief one—the acceptance of copulation—should serve as an indication of this time. In many mammals oestrus and ovulation occur on the day of parturition. In view of these facts, if access to the male were not permitted until the second day following parturition, the length of the oestrous cycle would be determined by the interval between this post-partum oestrus and the next acceptance of coitus. Furthermore, on the assumption that the cycles are of uniform length, for example, ten days, coitus should take place not only on the tenth but also on the twentieth, thirtieth, etc., day after parturition. As a matter of fact, an attempt was made by one of us to determine the cycle in the rat and mouse on the basis of the above assumption. A large number of careful observations were made, but when these were analyzed no system pointing to a sufficiently uniform cycle could be discovered.

Simplification of this method was then attempted in the use of vasectomized males by which it was sought to avoid the complications of pregnancy, but some of these complications were introduced by the mating act itself, as this paper will disclose. These studies gave as discordant results as did the former ones!

In still another effort a series of animals was killed according to a schedule, and the ovary, itself, including the oviduct, thus examined in serial sections at precise and frequent time intervals after parturition.

The present monograph will make evident how variations in the oestrous cycle itself and the inadequacies of method inevitably prevented recognition of the true cycle by the employment of any of the above devices.

For somewhat over thirty years scattered papers in the literature have indicated that periodic changes can be recognized in the structure of the mucosa of the generative tract in mammals, but no systematic study of these phenomena with a single recent exception seems to have been attempted. We refer to the papers of Moreau, Lataste, Retterer, and Königstein. Yet even though this older literature made it seem extremely probable that these changes were associated with the sexual history of the animal and in particular with oestrus, and were hence definitely related to ovulation, it was clearly impossible to follow these changes in any one animal, or even to predict when they would recur, unless possibly advantage was taken of their relation to some outspoken event such as pregnancy. By the fortunate discovery that in the guinea pig these mucosal transformations are accompanied by the dehiscence of epithelial cells so that at times the lumen of the vagina has a characteristic cell content, it has been possible for Stockard and Papanicolaou to show us that we may discover with ease in the living animal the exact occurrence and progress of these cycles. When it has been proved, as Stockard and Papanicolaou have done for the guinea pig, and as we have been able to do with exactitude for the rat, that these cycles are correlated with the rhythmic discharge of ova from the ovary, it will be seen that we now have in our hands for the first time an accurate method for the detection of ovarian function in experimental animals. This fact promises important consequences, for it enables us to investigate disturbances of ovarian function which may be experimentally produced.

Before proceeding to such studies, it would be necessary to establish clearly all the characteristics of what we have called the normal oestrus or reproductive cycle in the animal form which we have chosen to investigate. The present monograph is devoted to that study. We may remark at once that the rat, while exhibiting certain fundamental similarities when compared with the guinea pig, has also striking differences. It is hoped that this inquiry will serve as a preliminary to establish some of the more fundamental phenomena which may be expected to characterize the various steps in the oestrous cycle throughout the higher mammalia. It has been carried on by us for a period of some four years and has been conducted with sufficient deliberation and repetition to lead us to cherish the hope that we have been able to establish reliable generalizations. It has involved inquiries into the sexual behavior of about a thousand individual animals, upon each of which, for the period of observation or experiment, accurate daily records have been kept. No one who has not experienced a similar self-imposed, long-continued, and meticulous responsibility will readily appreciate the amount of conscientious concern necessary to such tasks. Well over five hundred careful autopsy protocols have been founded on the study of complete serial sections of ovarian and representative sections of vaginal material.

Grateful acknowledgment is made of the grants awarded by the Board of Research of the University of California and the American Medical Association for the prosecution of this work.

II. LITERATURE

In view of the excellent work of Marshall on the *Physiology of Reproduction*, a comprehensive review of the very extensive literature on this subject is unnecessary. However, for convenience, a list is presented of the times of oestrus and ovulation which have been reported for various mammals by various observers:

Animal	Length of Dicœstrous cycle	Ovulation	Authority	
CARNIVORA:				
Cat		Dependent on copulation	Coste (Hansen)	1847
	14 days		Marshall & Jolly	1905
		Only at copulation	Ancel & Bouin	1909
			Bouin & Ancel	1909
	Fraction of gestation	Only after copulation and independent of abortion	Winiwarter & Saimont	1909
	About 14 days		Marshall	1910
		Only after copulation	Longley	1910–11
		Only after copulation	Van der Stricht, R.	1911
		Only after copulation	Hammond & Marshall	1914
Dog		Spontaneous	Spallanzani (Heape)	1786
		Spontaneous at heat	Bischoff	1844–5
		Spontaneous	Sir E. Millais	1884
		Spontaneous	Pierre Rossi	1884
		Spontaneous (?) at heat	Iwanoff	1900
		Spontaneous at œstrus	Marshall & Jolly	1905
		Spontaneous and associated with rut	Ancel & Bouin	1908
		Spontaneous	Ancel & Bouin	1909
		Spontaneous	Marshall	1910
		Spontaneous at œstrus	Hammond & Marshall	1914
Lioness	3 weeks		Marshall & Jolly	1905
Otter	Month		Marshall & Jolly	1905
			Marshall	1910
MARSUPIALIA:				
Dasyurus		Independent of copulation	Hill	1910
		Spontaneous, after œstrus	Hill & O'Donoghue	1913
Didelphys virginiana		Spontaneous	Hartman	1916
				1919
RODENTIA:				
Ferret		Usually only with copulation, although sometimes spontaneous at œstrus	Marshall	1904

Animal	Length of Dicestrous cycle	Ovulation	Authority	
Guinea Pig	Irregular	Spontaneous within 24 hrs. pp.	Bischoff	1852
	38, 43 & 44 days (Hensen)	During first 24 hrs. pp. May be spontaneous, but greatly influenced by copulation	Reichert	1861
		Spontaneous, but may also be influenced by copulation	Bischoff	1870
	18 (17, 18, 35, 37) days	Spontaneous, also 6-10 hr. pp.	Hensen	1875
		Ovulation and copulation coincident	Rein	1883
	15-20 days (?)		Retterer	1892
	10-20 days	At rut	Lataste	1892
		Spontaneous	Iwanoff	1900
		Spontaneous after parturition	Lams & Doorme	1905
		5-20 hrs. pp. Vagina open only at heat, and ovulation when vagina opens	Rubaschkin	1905
		Spontaneous after parturition	Sobotta	1906
	18½-24 days	Spontaneous 6-10 hrs. pp.	Loeb, L.	1909–11
		Only at copulation	Bouin & Ancel	1909
		Only at copulation	Ancel & Bouin	1909
		Not independent, exceptional without copulation	Bouin & Ancel	1910
		Spontaneous (?) 12-17 hrs. after parturition and 2-4 hrs. after copulation	Lams	1913
	16 days		Loeb & Hesselberg	1917
	16 days	Spontaneous	Stockard & Papanicolaou	1917
Mouse	10 (or 20) days	Spontaneous. Copulation only after ovulation	Lataste	1883
	Copulation at fairly equal intervals		Tafani	1889
	10 days		Morau	1889
	10 days	At rut. Spontaneous (?)	Lataste	1892
	21 days	Spontaneous	Sobotta	1895
	10 days		Heape	1900
		Spontaneous	Lams & Doorme	1905
		Spontaneous	Gerlach	1906
	Oftener than once during (21) 30 days	Spontaneous	Melissinos	1907
		Spontaneous after parturition	Kirkham	1907
		Only at copulation	Bouin & Ancel	1909
		Spontaneous after parturition	Kirkham	1910
		Spontaneous after parturition	Long & Mark	1911
	17½-18 days	Spontaneous	Long & Smith	1916
	16½-19 days	Spontaneous	Smith	1916

Animal	Length of Dicestrous cycle	Ovulation	Authority	
Rabbit		72 hrs. after copulation	De Graaf	
		Eggs in oviduct 3 da. p. coitum	Cruikshank	1797
		9-10 hrs. after copulation	Barry	1839
	38, 43, 44 days	Spontaneous or 9-10 hrs. p. coitum	Bischoff	1842
		9-10 hrs. after copulation	Reichert	1861
		Spontaneous after parturition	Weil	1873
		May be induced by copulation		
	35, 37 days	Induced by copulation	Hensen	1875
		About 12 hrs. after copulation		
		8-10 hrs. after copulation	Van Beneden	1880
		Spontaneous at heat immediately after parturition	Ott	1882
	No periodicity	Ovulation and copulation coincident	Rein	1883
	15-30 days (?)		Retterer	1892
	About a month?		Lataste	1892
		Only at copulation, 10 hrs. after	Heape	1897, 1905
		Spontaneous pp.	Iwanoff	1900
	10-21 days. Average 15 days. Very variable		Heape	1900
		7-10 hrs. after copulation	Regaud & Dubreuil	1908a
	30 days ?	Occasionally spontaneous. Presence of male, without copulation does not cause ovulation	Regaud & Dubreuil	1908c
		Only at copulation	Bouin & Ancel	1909
		Only at copulation	Ancel & Bouin	1909
	Shortened by presence of male	Only at copulation. Hastened by mating	Dubreuil & Regaud	1909
	10-15 days (Heape) even three weeks	Usually only at copulation, but sometimes spontaneous	Hammond & Marshall	1914
Rat, white		Spontaneous at rut?	Bischoff	1844
	10 days		Morau	1889
	10 days	At rut, spontaneous	Lataste	1892, 1893
	10 days		Heape	1900
	14 days		Königstein	1908
		Spontaneous after parturition	Burchard	1910
		Spontaneous after parturition	Kirkham	1910
brown	5 days		Miller	1911
white		Spontaneous after parturition	King	1913
	21 days (?)	Spontaneous	Kirkham & Burr	1913
	10 days	Spontaneous	Long & Quisno	1916
	5 days (average)	Spontaneous	Long & Evans, present paper	1922
Dipodillus	8, 9-10 or 14 days	At rut	Lataste	1883
Rodents in general		Only at copulation	Ancel & Bouin	1909
	10 days, even 5 days	At rut	Lataste	1887, 1893
Muridae (rats, mice, Gerbillus, Meriones)	10 days	At rut	Lataste	1892
Muridae (Eliomys, Gerbillus, Dipodillus (2 sp.), Meriones (2 sp.)	10 days		Lataste	1887

Animal	Length of Dicestrous cycle	Ovulation	Authority	
"Laboratory rodents"	3-4 weeks		Königstein	1907
UNGULATA:				
Cattle	3-4 weeks		Ellenberg	1892
	19 days in summer			
	20-21 days in winter		Wallace	1876, 1904
Cow	3-4 weeks		Fleming	1878
		Spontaneous, at heat	Iwanoff	1906
		Spontaneous	Bouin & Ancel	1909
	21 days		Williams	1909
		Spontaneous at œstrus	Marshall	1910
		Spontaneous at œstrus	Hammond & Marshall	1914
	2nd day of 21 day cycle	Spontaneous	Kupfer	1920
	21 days	Spontaneous	Zietzchmann	1921
Sheep		Only after copulation	Hausmann	1840
		Spontaneous at rut	Bischoff	1844 a, b
	2-4 weeks		Fleming	1878
	17 days or 3-4 weeks	With or without (?) copulation	Bonnet	1884
	20-30 days		Ellenberger	1892
	15-16 days	Spontaneous at earlier periods but not at later. Hastened by copulation	Marshall	1901–3
		Spontaneous at rut	Iwanoff	1906
Sheep Lombardy	13-21 days		Marshall	1910
		Spontaneous at rut	Hammond & Marshall	1914
Sow		After copulation	Hausmann (Marshall)	1840
		Spontaneous at rut	Bischoff	1844
	2-4 weeks		Fleming	1878
		Spontaneous	Bouin & Ancel	1909
	2-4 weeks	Spontaneous, at œstrus (probably)	Marshall	1910
		Spontaneous at rut (certain)	Corner & Amsbaugh	1917
	18-23 days (mean 21 days	4-9 days pp.	Struve	1911
		Spontaneous at rut	Corner	1917
	21 days	Spontaneous	Corner	1921
Wild cattle, deer, and other similar forms in captivity	3 weeks		Heape	1900
Donkey		Spontaneous at heat	Marshall	1910
Mare	2-4 weeks		Fleming	1878
		Spontaneous	Heape	1897
		Spontaneous at heat	Iwanoff	1906
		Hastened by copulation	Dubreuil & Regaud	1909
		Spontaneous	Bouin & Ancel	1909
	About 3 weeks	Spontaneous at œstrus	Marshall	1910
		Spontaneous at œstrus	Hammond & Marshall	1914
All animals		Spontaneous	Pouchet	1847

It will be noticed that ovulation is spontaneous at rut in all except the cat, ferret, and rabbit, and, in the latter two, spontaneous ovulations are recorded. The list shows also that, while the oestrous cycle is known in some of the larger animals, and in some cases the exact relation between oestrus and ovulation, it is in the guinea pig only, among laboratory animals, that the oestrous and ovulation cycle has been carefully worked out. It is interesting to note the ten-day cycles given for the rat and other rodents by several investigators and especially the variable period given for Dipodillus by Lataste.

"The Changes that Occur in the Non-Pregnant Uterus during the Oestrous Cycle" forms the subject of a chapter of Marshall's *Physiology of Reproduction,* which summarizes the researches on the larger mammals including man. Very little space, however, is given to the rodents, not even his own work on the ferret being cited. Probably lack of room prevented any mention, more than a single sentence, of vaginal changes, especially since most published accounts deal with the pregnant animal. There are, however, several papers on changes in the vaginal mucosa at rut and during pregnancy.

In the earliest of these, Morau (1889) describes the vaginal mucosae, occurring at rut, of mice killed at intervals after copulation. The periods of rut recur at intervals of ten days except during lactation and pregnancy. At the time of copulation or rut the epithelium is stratified and consists of three distinct parts, the outermost of which is cornified. By the fourth day after copulation the cornified layer has become loosened and completely lost. If the animal is pregnant, the cornified layer is apparently not re-formed until the next rut after parturition. In animals which fail to become pregnant, the condition at copulation may recur on the eighth and sixteenth days, and one case is recorded of desquamation on the fourteenth day. Morau states that he finds the same conditions in the rat (both black and albino) at rut, and that the vaginal epithelium undergoes changes in the guinea pig, rabbit, one of the species of Gerbillus, and all rodents. Although Morau's work was done primarily on pregnant mice, it is clear that he has observed a cyclic change which is independent of pregnancy. His observations being confined to sections, it was not possible for him to follow the cycles in one animal. Like Lataste, he thought that the desquamated epithelium formed a "vaginal envelope."

In 1892 and 1893 several papers were published by Lataste and by Retterer on the periodic transformations of the vaginal epithelium of rodents. Retterer's first paper is concerned chiefly with the conditions of the epithelia in the dog, cat, sheep, and pig during pregnancy. He thinks that rut and copulation do not influence the mucosa, but that the last days of gestation and parturition do in carnivora and ruminants.

In a second paper (1892*b*) Retterer describes the changes occurring in the vaginal mucosa of the non-pregnant guinea pig. The animals were killed at

intervals during the first twenty days following parturition. For the first fifteen days the superficial cells are mucous, but at the fifteenth day there is formed under the superficial layers a cornified stratum which is easily detached during manipulation. A similar cornification occurs in the dog at rut. In the cat it is stratified but not cornified, while in the non-pregnant rabbit the mucosa becomes greatly thickened and stratified.

Lataste (1892–1893) points out that his own and Morau's work show that the vaginal changes are associated with rut primarily rather than with gestation. In rodents (rats, mice, Gerbillus, Meriones) at the approach of rut the lips of the vagina become swollen so that it is possible to tell a day before when a female will copulate. This swelling also corresponds to a cornification of the mucosa. After rut the keratinized layers are lost, forming a vaginal envelope or, if a plug is present, the outer parts of the plug, as described in Lataste's papers. Lataste suggests that the cornification is a protection especially at the occurrence of copulation. There is, then, a rhythm in the vaginal mucosa, a temporary thickening at rut followed by a thin mucous-like epithelium during the interval.

Königstein (1907) describes the changes in the genital tract during pregnancy and at heat in several rodents used in the laboratory (rats, guinea pigs, and rabbits), also in a dog. Although he deals with changes in the vagina during pregnancy chiefly, he clearly indicates that in the non-pregnant rat the flattened epithelium is transitory, and that it is completely cast off and a new one formed four days after parturition. Such flattened epithelium may contain leucocytes even to the outer layers. Apparently it is gone after heat and is replaced by cylindrical epithelium.

The most recent papers on the subject of oestrus are the excellent ones on the guinea pig by Stockard and Papanicolaou (1917, 1919). They were able, by inspection of the vagina and by microscopic examination of smears, to determine the time of oestrus and ovulation, and the length of the cycle. They describe also the histological changes in the vagina, uterus, and ovaries. These are compared in some detail farther on (p. 48) with those in the rat.

III. METHODS

A satisfactory conception of physiological phenomena in this field cannot be obtained from a miscellaneous material of unknown or greatly varying age and of uncertain past sexual history. In such studies the greatest emphasis must be laid on the necessity of securing individuals in full reproductive vigor, conditions which are obtained with most certainty, of course, by the establishment and careful control of an animal colony.

The rats used in this study were both white and colored, descendants of a cross made about seven years ago between several white females and a wild gray male caught in Berkeley, black, gray, and hooded varieties resulting. It is a vigorous stock due in part, no doubt, to the cross, for one of the original hybrid females had her last litter when nearly two and one-half years old. As far as our experience goes, there is no difference to be observed between these different colored rats with respect to the oestrous cycle.

The colony has been fed with table scraps supplied daily from a large hotel, thus insuring them a variety of nutritious food. In the case of animals used for experimental purposes this diet was supplemented by whole milk and occasionally by other substances, such as uncooked liver, greens, etc. Although the recent wealth of studies in nutrition makes it no longer necessary to speak of the necessity of a generous and varied diet of both the correct nutritive and vitamine consistency, we may be pardoned for emphasizing it by at least this brief mention because we have been able to satisfy ourselves of the effect of a poorer nutritional régime in consequent irregularity of the sexual cycles. It may be questioned whether table scraps of notorious variability in food value could prove an ideal food. One should eliminate the fluctuations which will characterize any but well standardized diets.

We feel that it is necessary also to dilate upon the great importance of daily and regular care, rigorous cleanliness, and access of the individual animals to adequate space and air. We have observed repeatedly in attempts to rear considerable numbers of animals in single cages that, though the space seemed more than adequate and the ventilation through open meshwork all that could be desired, the gregarious habits of the rodent actually prevented these needed desiderata from being obtained. These animals tend habitually to sleep in piles, even in warm rooms where the need for conserving body temperature could not exist. Though it may seem doubtful that we could explain the poorer state of a colony maintained in this way by respiratory inadequacy, it is nevertheless true that separation of animals into pairs or at most into fours in smaller unit cages has in our hands proved to be the only dependable method of insuring vigorous stock. It may be thought also that these rodents would be able to maintain perfect health in the presence of very considerable fluctuations of temperature, represented by diurnal or seasonal climate, but in our opinion such is not the case. They thrive so much better under conditions of equable temperature that we have taken the pains to install an electric source of heat and, providing the appropriate supply of fresh air is at hand, we have seen only good effects with the maintenance of a temperature from 65° to 68° F. This is especially true where animals are "naked" in the cages, so to speak, that is, where one does not install elaborate nest materials and separate nest boxes—things of great nuisance to handle and to clean and which we have learned to dispense with entirely.

A first hand acquaintance with rats of some years' duration has emphasized to us further the inestimable advantages of direct and frequent handling of his stock on the part of the investigator. We do not hesitate to refer the formidable attitude of aggression on the part of this animal, of which bacteriologists and others speak, to its manipulation with tongs or other rough devices. If grasped with the hand so that the head may not be turned to inflict a bite and if handled gently, these animals may be controlled with surprising ease. We do not indeed handle them in any securer fashion in inflicting needle wounds in intraperitoneal injections of vital dye, and we dwell upon the matter because the infliction of pain or of unnecessary fright and the consequent retaliation resulting therefrom might lead other investigators to give up the hope of pursuing that intimate acquaintance with the individual animal which is demanded in an investigation of this sort. An unaided observer will have no difficulty in holding a gentle animal with one hand and with the other introducing a small speculum into the vagina or taking samples of the vaginal content by means of the introduction of a pipette, spatula, or other instrument. No natural combativeness on the part of the rat is known to us except the defense exhibited by a suckling mother. The female with young will not hesitate to protect her brood, but, if driven from them—and her tendency to alarm makes this easy—she may be grasped by the tail and removed from her cage. Once out of the accustomed surroundings and mastered by being grasped, as is usual, behind the shoulders, a speedy change in her psychology ensues and she may be handled further with perfect impunity.

A final word on method may be allowed in the description of a device (fig. A) for preventing suckling of a newborn brood. We have found it expedient to place the mother in an "obstetrical" compartment with an inclined or slanting floor which has a small opening at its lower edge. In our experience, this device does not permit much time to elapse before the newborn young roll out through the slit arranged at the lower end. Normally, even if the period of suckling has been only a few hours long, this nevertheless shows itself in the young in the peculiar white spot created by the milk-filled stomach which shimmers faintly through the body wall. Young delivered from the floors of these compartments do not appear to have succeeded in suckling at all. This device was essential for us, as will be shown later, when we sought to establish the effect of lactation on ovulation and on the corpora lutea.

IV. GROSS ANATOMY OF THE REPRODUCTIVE ORGANS

The essential relations of the reproductive organs in the rat are shown diagrammatically in figure 1. The ovary lies within a small, completely enclosed space, the periovarial space, the walls of which are formed chiefly of the periovarial membrane. The periovarial space has no connection with the peritoneal

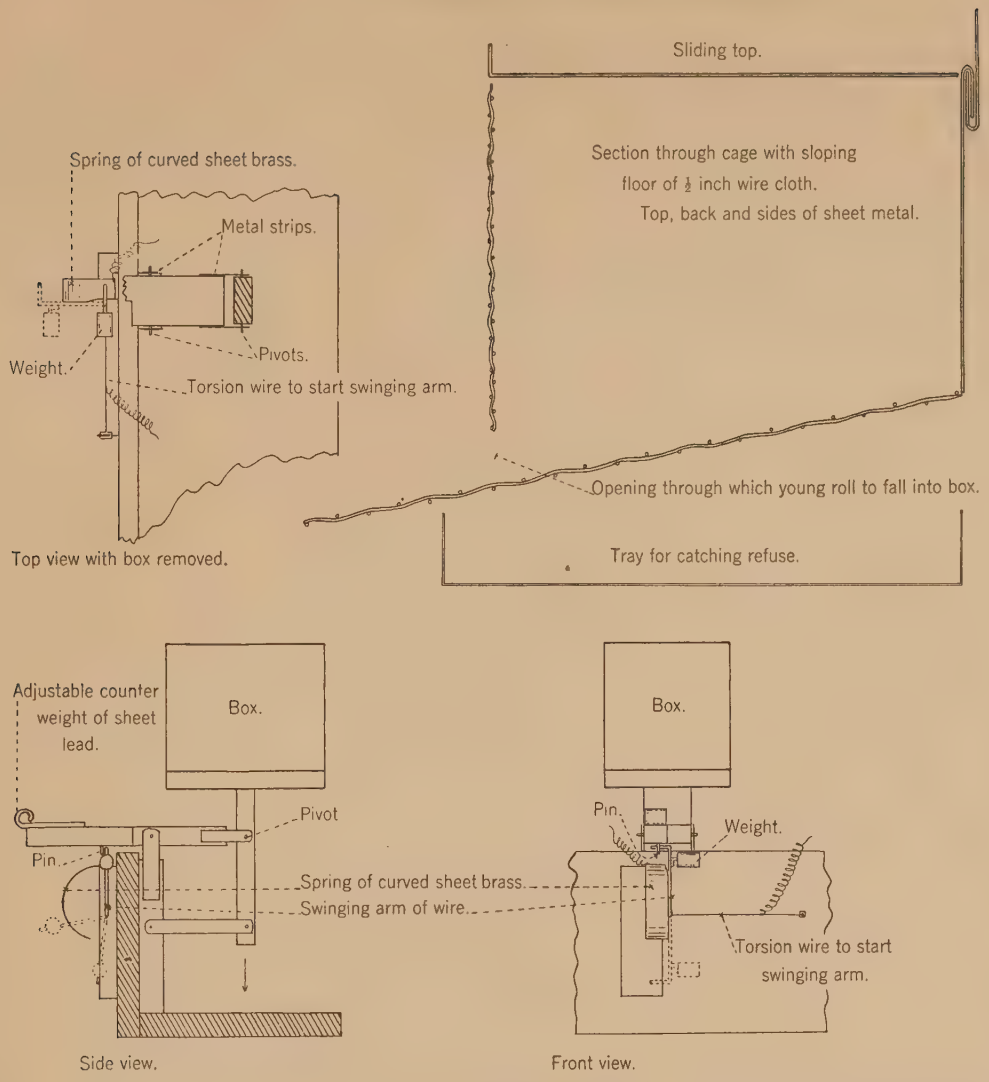


Fig. A. Apparatus to prevent newborn rats from being suckled and to record automatically the time of their birth. This figure represents the essentials of one unit of the apparatus, part of which is a chronograph with a drum revolving once in 24 hours. As many units may be operated as desired. The pregnant female is placed in the cage, which offers ample room and from which she is removed daily for feeding. Since nest material cannot be used, the cage must be kept in a room sufficiently warm.

The young as they are born roll out and drop into the box before the mother can suckle them, for without constant attention the young rats cannot be kept in the cage. The box rests on a balance so adjusted that a small addition of weight to the box depresses it in the direction of the arrow. The movement thus imparted to the balance raises the pin and releases the swinging arm which in its downward movement scrapes along the edge of the spring of curved sheet brass, making an electric contact. The current thus closed for a fraction of a second operates an electromagnet which through the medium of a pointer records its movement on the smoked paper of the drum. It is then a simple matter to determine the time of birth of the young rats (or mice).

In order that the swinging arm shall start without fail it is attached to one end of a fine spring brass wire, the other end of which terminates in a narrow rectangular loop. The loop passes over a post and is adjusted at such an angle to the arm that when the arm is raised into position the torsion wire is twisted enough to keep the upper end of the arm against the pin and on release to propel it through a part of its arc of motion. The torsion wire also serves to keep the arm in contact with the spring of sheet brass. × ca. ¼.

cavity as shown by the distention of the membrane by a characteristic fluid at the time of ovulation. The oviduct is thrown into eight to ten folds which may be divided into two groups, one the distal, the other the proximal group. The proximal group, according to Huber, is capable of still further subdivision as

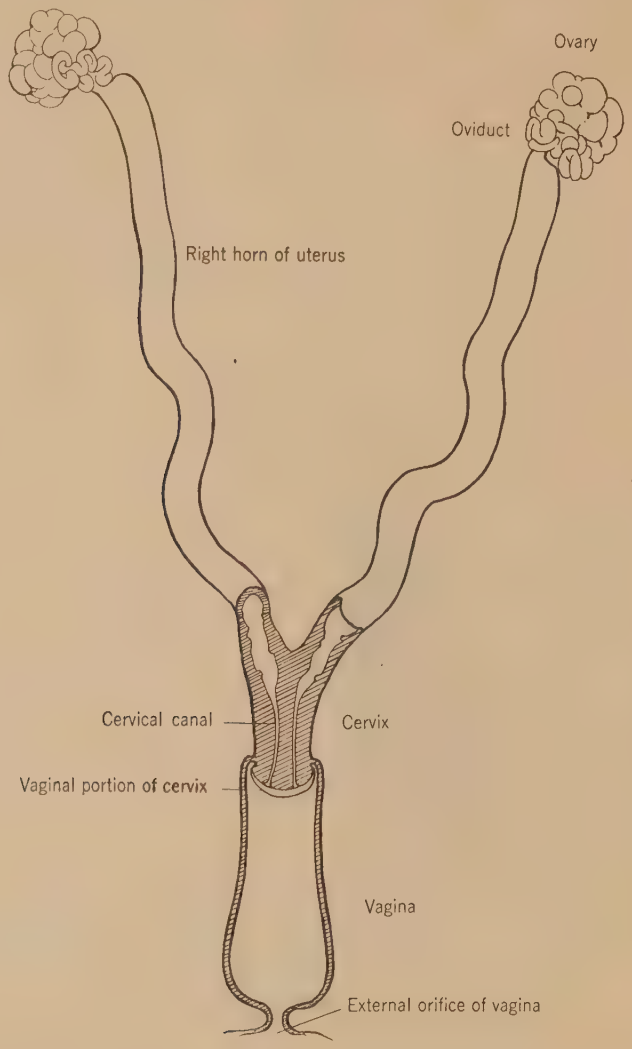


Fig. 1. Semidiagrammatic ventral view of the reproductive organs of the female rat partly laid open by a cut in the frontal plane. The lumina of the two uterine horns communicate with the vagina independently through the two cervical canals which open in the tip of the cervix protruding into the vagina. For details of oviduct and ovary see figures 38 and 39, pages 27 and 28.

Sobotta has described for the mouse. The distal group communicates with the periovarial space through the club-shaped, ciliated end. The folds of the distal group differ from those of the proximal in being very freely movable, in having

thin walls allowing of great distention, and in having the mucosa thrown into high ridges and covered with distinctly ciliated epithelium. The proximal part of the oviduct does not open into the exact tip of the uterine horn, but, as Fischel has described, through a papilla slightly lateral to the tip.

Although the walls of the uterine cornua are fused in their lowermost portions, their lumina are entirely independent of each other, opening into the vagina through separate orifices. These external orifices of the cervical canals are difficult to detect among the lappets of the vaginal portion of the cervix. Their position is indicated by bristles in figure 2, plate I.

V. THE NORMAL OESTROUS CYCLE

A. Changes in the Vagina

1. OBSERVATIONS ON THE LIVING ANIMAL

If the vaginal mucous membrane of rats in full reproductive vigor is examined daily by means of an appropriately small speculum, two strikingly different conditions will be encountered from time to time. These conditions, which alternate with each other, may be roughly described as the "moist, pinkish," and as the "dry, white" conditions, and either condition will usually be found to recur in four days. With the short cycle of four days, which we have found to be normal for the majority of young adults, it accordingly happens that during about one-half of this time the vagina has the moist, pinkish appearance characteristic of the dioestrous pause and during the other half the "dry" condition associated with a succession of events grouped together and related to oestrus proper. The moist, pinkish condition typifies the dioestrous pause, or the interval between oestrous changes. The dry, lusterless, or white condition is usually associated with a swelling or turgescence of the small radiating folds about the vaginal aperture (fig. 3, pl. I). In its incipiency it is always associated with the manifestation of oestrus, and toward its close with the occurrence of ovulation.

Vaginal Smears.

These stages succeed one another in orderly sequence, and each is characterized by a different histological make-up of the vaginal fluid. It is indeed not the least remarkable of the histological phenomena encountered in these studies that we should have so clearly marked a succession of the cell types which are thrown off within the vagina. Our study has given us a satisfactory explanation of the origin of these cells and has also in a sense made it clear why we should have this succession, but they have only served to make more precise our acquaintance with the astonishingly orderly and steplike character of the

cellular dehiscence, by means of which there appears in the vaginal lumen at any one time only one type of epithelial cell. These studies have shown us that the dehiscence of the epithelium, which proceeds relatively speedily, also takes place at about the same rate over the entire mucosal surface, for were it otherwise we would not have the homogeneous cell picture which we actually find, but only confusion arising from the occurrence in some localities of an earlier, and in others of a later, succession of the same events.

We have chosen the stages disclosed by change in the content of the vaginal smear as of fundamental value in our study—as the stages with which to correlate changes in the histological structure of the internal organs of reproduction for two weighty reasons:

(1) In contrast to other phenomena (e.g., the appearance of external swelling and, in particular, the duration of oestrus), each of the stages marked by changes in the vaginal smear is approximately constant in length in all animals.

(2) The "vaginal stages" constitute the only reliable method of recognizing subdivisions of the oestrous cycle in the living animal.

We have, accordingly, made the histological characteristics of the vaginal smear the criteria for delimiting steps in the oestrous cycle and for this reason, before giving an account of the changes undergone by the generative apparatus in each stage, shall begin with a description of the successive histological pictures found at each stage of oestrus in the vaginal smear. (See table 1, p. 42.)

Dioestrous Interval.

During the interval, or dioestrous pause, which constitutes about half of the entire cycle and which possesses a relatively clean, moist, glistening, normally somewhat translucent, pinkish mucosa, the pipette, spatula, or other sampling instrument will succeed in withdrawing from the vaginal lumen a variable quantity of thin, somewhat stringy mucus in which are entangled leucocytes and small, irregularly shaped, free epithelial cells (figs. 4 and 5, pl. II). Both types of cell may occur in considerable numbers in some animals or may be both of them very sparse in others. The leucocytes are usually fairly abundant and are the characteristic small, polymorphonuclear elements which, as is well known, often have annular nuclei in the rat. The leucocytes are often distorted in one dimension so as to be slightly elongated within the "strings" of mucus. The epithelial cells are always single, never in groups.

Stage One.—Oestrus is inaugurated by the occurrence of a distinct stage which we have designated as "Stage One" (the stage corresponding to Heape's pro-oestrum). While characterized by a distinctive histological picture of the vaginal content, it may also often be detected macroscopically by the changed appearance of the surface of the vaginal mucosa as disclosed by the speculum;

the mucosa is no longer moist, seems distinctly less transparent, and, while possessing a slight sheen, or luster, has a characteristic opaque look. In some animals a further external characteristic of Stage One may be found in an increasing turgescence of the small radiating folds about the vaginal aperture (fig. 3b, pl. I) as compared with the condition during the dioestrous interval (fig. 3a). To the naked eye, however, these appearances are not so distinctive that they may not be overlooked, but under no conditions will the incidence of Stage One escape the observer who takes the precaution to obtain a microscopic sample of the contents of the vaginal lumen. To a small drop of physiological saline, Ringer's, or Locke's solution, a narrow spatula may be applied after its withdrawal from the vagina where it has been in contact with the mucosal surfaces. This simple method of sampling the vaginal content is actually adequate for an infallible diagnosis even when studied with a lens which gives a magnification of but eighty diameters. The small translucent, jelly-like, adhesive mass withdrawn on the tip of the spatula is not easily dislodged into the drop of saline solution upon the slide. In it leucocytes have entirely disappeared and great numbers of small, round, nucleated epithelial cells of strikingly uniform appearance and size are now present (figs. 10 and 11, pl. II). Occasionally small sheets of these cells may be encountered. The microscopic picture is absolutely characteristic and unmistakable, for these particular cellular elements have occurred and will occur at no other time in the oestrous cycle. The manner of their formation as a peculiar layer at the surface of the vaginal epithelium will be disclosed later. Not the less striking and unexplained is their sudden dehiscence, a fact correlated with the equally sudden cessation of leucocytic migration. Leucocytes, indeed, are not encountered again until the close of the next two stages in oestrus. Stockard has found a similar behavior on the part of these cells during the proestrum and oestrus in the guinea pig and it would appear that this peculiar lull, or sharp pause, in the penetration of the mucous membranes by leucocytes, which is otherwise going on constantly throughout the dioestrous pause, is a very fundamental characteristic of the histology of oestrus in the two rodents which have been investigated. The average duration of Stage One is twelve hours.

During Stage One females will usually not accept copulation, defending themselves against any aggression on the part of the males. It is hence proper to recognize this as the stage which is normally preparatory for oestrus. In some cases, however, at about the middle of the stage, and in still more cases toward the end of it, animals will mate, but even under these circumstances they do not usually show oestrous excitement in the characteristic manner typical of the following stage. As will be described at some length below, the acceptance of coitus can easily be tested by employing a small number of young accustomed males in a well illuminated, flat cage which can be instantly opened in order to interrupt the act.

Stage Two.—In Stage Two the macroscopic changes which we had noted as characterizing Stage One, notably the beginning swelling of the vaginal lips and the dry mucosa, are now increasingly evident. The speculum or spatula meets notably more resistance at attempted introduction into the vagina. An equally definite change takes place in the microscopic character of the vaginal smear, for the small, nucleated, and somewhat granular appearing epithelial cells characteristic of Stage One are rather suddenly replaced by large, thin, transparent, non-nucleated, scalelike elements—the cornified cells (figs. 21 and 22, pl. II). For this reason Stage Two may well be designated as the "cornified cell stage." The sample, while still scanty in amount, is opaque, whitish, and granular, the crumbling particles being easily tapped off into the drop of saline, where they have a tendency to float. There is still a singular absence of leucocytes, whose sudden disappearance was so marked a characteristic of Stage One. It is during the beginning of this stage that the female usually shows unmistakable signs of heat. If placed within a mating cage and, through some chance, not approached by the males, she will manifest what we have been led to recognize as oestrous excitement by quick, darting motions, or hops, with the back arched, with occasional quivering of the body and with a curious shaking of the ears. We may state that this behavior, never encountered at any time other than oestrus, need not, however, invariably be manifested by individuals in heat. A simple and unmistakable test of heat is furnished by the attitude of the female on the approach of the males. Mating is made possible by a characteristic flattening of the back, or slight opisthotonos. During this period there is usually a disagreeable odor attached to the vaginal secretion, which perhaps is a means of attracting and exciting the male.

Stage Three.—Stage Three, which may be equally well named "the late cornified cell stage," cannot be separated abruptly from Stage Two, the histological picture of the vaginal smear being identical although an exaggeration of that characterizing the preceding stage. Indeed, the accumulation of cornified, non-nucleated epithelial plates within the lumen of the vagina now proceeds so rapidly that easily visible masses of whitish, granular, or pasty substance always occur deep in the vagina near the cervix. These masses consist exclusively of enormous numbers of the elements in question without admixture with other cells. One who is familiar with the character of the "plug" (*bouchon vaginale*) which the male rat leaves in the vagina after coitus will at first easily mistake these white masses for fragments of the "plug". The most superficial microscopic examination, however, shows that they are made up of sheets or of single cornified elements whereas the male secretory product is amorphous. But besides this accumulation of cheesy substance, macroscopically evident within the vagina, Stage Three is further typified by a very important characteristic in the

fact that animals in this stage will usually no longer accept coitus.[1] The average combined length of Stages Two and Three would appear to be about thirty hours. Throughout this period the vagina is dry.[2] The swelling of the vaginal orifice may also persist.

Stage Four.—Stage Four, the metoestrum or the leucocyte-cornified cell stage, is inaugurated by the appearance in the vaginal smear of leucocytes among the cornified cells (figs. 31 and 32, pl. II) and ends with the disappearance of the latter. The leucocytes cause a softening of the granular masses seen in Stage Three and convert them into a substance of a cheesy, creamy, and increasingly fluid consistency. Before the cornified cells completely vanish from the smear, epithelial cells reappear so that during a short interval all three cellular types are present (cornified, non-cornified, and leucocytes). This ushers in the beginning of the dioestrous pause, which may be recognized by the complete disappearance of cornified elements so that the smear again consists of leucocytes and epithelial cells. Stage Four is normally of about six hours duration and might well be known as the stage of transition to the resting condition. It is chiefly characterized by the sudden resumption of leucocytic migration.

It is interesting that in a certain proportion of animals the epithelial desquamation of the mucosa may proceed somewhat further than to the cornified cell layer before leucocytes come in, and hence we may have the cheesy state, Stage Three, immediately succeeded by a few hours of a stage in which rather large, spindle-like, nucleated epithelial cells are shed. In such cases, however, vestiges of the cornified cell are always present and when leucocytes appear three cell types are actually found.

Since there is thus an invariable succession of cell types occurring in the vaginal smear at various times in the oestrous cycle, the stages in the cycle may with equal propriety be named from the cell content of the vaginal smear. Should we do this, we could designate them as the *stage of the sudden appearance of masses of uniform sized, nucleated epithelial cells dehisced from the surface, the stage of few large cornified cells, the stage of extremely abundant cornified cells, the stage of many leucocytes admixed with cornified cells,* and, finally, in the dioestrous pause, *the stage of leucocytes with scanty epithelial cells.*

2. CORRELATED HISTOLOGICAL CHANGES

Histological changes in every portion of the reproductive tract are correlated with the above stages which we have characterized by means of the vaginal smear (see table 1, p. 42). For obvious reasons we shall begin with a description of the histology of the vaginal mucosa, the changes in which explain so

[1] However, see the description of length of oestrus on page 33.

[2] Except for the very brief period of time which may immediately succeed sudden emptying of the uterus and drainage of its fluid into the vagina.

beautifully the characteristics of the vaginal smears. Our description refers to the histological conditions occurring in the mucosa of the vaginal folds at, or near, the cervix for the reason that:

(1) There can be no uncertainty concerning the position of the region in question.

(2) Possible injury caused by examination with the spatula is least likely to occur so deeply.

(3) It must be confessed that, in spite of the remarks which have been made previously regarding the singular synchronism in epithelial dehiscence, the changes do not actually take place at exactly the same rate or to the same degree in all parts of the vagina, even though they tend to do so.

To begin with, we may summarize the main histological events occurring in the vaginal mucosa by stating that the mucosa is depleted to a very low layer during the resting period, or dioestrous pause, the epithelium grows rapidly as Stage One (the pro-oestrum) approaches, and with its growth undergoes a differentiation into (1) a surface layer, (2) a subjacent stratum corneum, and (3) immediately subjacent to the latter, a small, but distinct, stratum granulosum beneath which are (4) several cell layers of the rete mucosum, and finally (5) a basal layer or stratum germinativum. This highly differentiated structure falls asunder during the active stages of the oestrous cycle (stages 1, 2, 3, and 4) by successive dehiscence of its various layers.

Dioestrum.

During the dioestrous interval the vaginal mucosa (fig. 6, pl. III) is thin, consisting of from four to seven simple cell layers, the squamous transformation of its upper cells being only slight. It remains constantly infiltrated by a certain number of leucocytes and constantly decreases in thickness through dehiscence of its superficial cells, a process evidently not completely compensated for by the scanty mitoses in the basal layer. Near the end of the dioestrous pause, however, these mitoses increase greatly in number and dehiscence almost ceases (fig. 7, pl. III). The epithelium, consequently, becomes higher (8 to 9 cells) and a squamous transformation of it becomes expressed. The actual surface cells, however (constituting a layer one to three cells deep), do not become cornified but become transformed in a characteristic way by swelling and other changes so as to form a well defined superficial layer, the layer of first stage cells. Coincident with this, squamous changes have advanced and immediately beneath the surface layer noticeably large, flattened, though nucleated elements are found. These are destined to undergo a further transformation into cornified cells, as later stages disclose.

Stage One.—In Stage One, or the pro-oestrum, the peculiar surface cell layer which we have described and which is unaffected by squamous transformation now reaches its fullest expression (figs. 12, 13, 14, pl. III), and begins to separate from the subjacent layers which themselves have undergone a striking differentiation. The epithelium as a whole has increased greatly in height, consisting of from nine to twelve cell layers, and, beneath its surface layer, now displays a well developed, strongly acidophilic stratum corneum and basophilic stratum granulosum. By the end of Stage One a complete detachment of the surface layer has ensued, a detachment which often occurs in sheets and which leaves the stratum corneum now exposed as the lining layer of the vaginal lumen.

The first appearance of the cornified layer actually beneath the surface of the epithelium is a remarkable histogenetic process noted long ago but not accurately described by Retterer and apparently lost sight of entirely by subsequent investigators. The French observer recognized the process in the guinea pig, dog, cat, and rabbit, and took care to note that in some cases cornification did not result, the essential fact being that, in the squamous transformation which ensues, the actual surface cells are exempted. Subsequent to our observations on the rat we availed ourselves of the opportunity to restudy the guinea pig, inasmuch as this feature had been overlooked by Stockard and Papanicolaou, whose model work marks so significant a step in our knowledge of this subject. The same process occurs in the guinea pig. Corner and Pelkan in this laboratory have also been able to confirm its occurrence in the rabbit.

Stage Two.—Stage Two, which it will be remembered is characterized by a vaginal smear in which cornified cells first appear, continues to show a high, stratified squamous epithelium (figs. 23 and 24, pl. III), which has its stratum corneum at the surface, dehiscence from which furnishes the cells of the smear. The well developed, desiccated stratum corneum aided by the considerable height of the epithelium doubtless causes the dry and lusterless appearance of the mucosa in the gross. The epithelium begins to be noticeably reduced in height by splitting and shedding of the stratum corneum which, after about twelve hours, takes place *en masse*. The epithelium is still free from leucocytes.

Stage Three.—Stage Three is characterized by the continued reduction of the vaginal epithelium, which is now from five to nine cell layers in depth, brought about chiefly through the complete detachment of the stratum corneum and stratum granulosum. This is the stage of accumulation of considerable cheesy or granular masses in the vaginal lumen and the masses consist exclusively of the cornified cells. Leucocytes have not yet entered the epithelium in any numbers.

Stage Four.—Stage Four, the vaginal smear of which is characterized by the appearance of leucocytes among the cornified cells, exhibits an extensive leucocytic infiltration of the vaginal epithelium which readily accounts for their

great numbers in the vaginal lumen (figs. 33 and 34, pl. IV). Few or no vestiges of the stratum corneum or granulosum can be found and nucleated squamous epithelial cells lie next the surface. The epithelium becomes progressively lower, being usually from four to eight cell layers in height toward the end of this stage.

B. Changes in the Uterus

Dioestrous Interval.

During the dioestrous interval the uterus is always slender (fig. 8, pl. I) and with a slitlike lumen, seldom exceeding 2.5 millimeters in diameter (varying from 1.6 to 2.5 millimeters). It is lined by a simple columnar epithelium (fig. 9, pl. V), which possesses next the lumen a delicate cuticular membrane. The character of this epithelium does not undergo any significant change during the early part of the pro-oestrum (fig. 20, pl. V).

Stage One.—No portion of the reproductive system shows so marked a response to the oestrous wave or one which can be more readily appreciated with the naked eye than does the uterus (see table 1, p. 42). Vascular engorgement, which expresses itself in the turgescence of the vaginal aperture, is also very evident in the uterus of animals killed in the pro-oestral stages (Stage One); toward the latter part of this stage there takes place an accumulation of clear fluid in the uterus which distends it to unusual proportions, to dimensions of 5 millimeters in diameter (figs. 16–19, pl. VIII). Whether or not the secretion of this fluid is comparable to the great formation of mucus which Stockard describes as characterizing the uterus of the guinea pig remains to be determined, but it is not unlikely that we have here analogous processes, the fluid in the guinea pig being different in consistency (mucus) and being constantly drained into the vagina, whereas in the rat it is a clear, watery, non-coagulable substance which distends the two uterine cornua tightly, the vagina remaining perfectly dry. This, indeed, constitutes one of the most striking differences between these two rodent forms. The accumulation of fluid within the uterus and the ensuing distention converts the columnar epithelium into a cuboidal one, although its height is not greatly altered. During this time there is no evidence of epithelial impairment and leucocytes are usually absent from the epithelium.

Although this oestral distention of the uterus by fluid is unique among mammals at present known to us, the production of fluid by the uterus during the time of hyperaemia is to be looked for in other forms. It is perhaps idle to speculate concerning the value of the retention of the fluid within the uterus of the rat, but it is impossible not to suggest that it may serve as a medium through which spermatozoa may swim rapidly toward the oviduct. If spermatozoa are immediately removed from the vasa deferentia of a male rat they will continue for a considerable time to exhibit motility when added to the fluid obtained

from the distended uterus at Stage One. It is a fact that enormous numbers of active sperm are found in the uterine fluid in an animal sacrificed a few hours after copulation.

Stage Two.—In the early part of Stage Two the uterus exhibits its most marked vascular congestion and reaches its greatest distention (fig. 25, pl. VIII); the distention disappears about the end of the stage. It will thus be seen that this behavior on the part of the uterus parallels closely the exhibition of oestrus on the part of the animal, which, as we have previously stated, may begin with the latter part of Stage One and is usually most evident in the early part of Stage Two. The uterine distention appears to subside rapidly. It is almost never encountered in Stage Three. In spite of this fact, we were at first inclined to look upon resorption as the probable mechanism for its loss because of the persistent dryness of the vagina during the whole of Stages Two and Three. We did not, however, reckon with the possibility that the discharge of uterine fluid may occupy but a very short time and that, if it be released suddenly, it could quickly drain through the vagina to the exterior and leave no evidence of its passage in this way unless animals were watched closely. For other reasons this duty eventually fell to our lot, a number of animals being under continuous observation at three-hour intervals, when by good fortune we actually observed the sudden appearance of fluid and leucocytes, most probably of uterine origin, in the vagina of an individual in Stage Two where the vaginal mucosa had been dry for six hours and resumed its characteristic dry appearance again three hours later.

Toward the end of Stage Two, when the uterine fluid has disappeared and the walls are flaccid, the normal character of the uterine epithelium reëstablishes itself, and the cuboidal type (fig. 26, pl. V) found at the time of maximum distention is succeeded by a columnar epithelium (fig. 27, pl. V). There may now begin a characteristic degeneration of the epithelium, which we have called vacuolar degeneration (fig. 27), because of the appearance and enlargement of cytoplasmic vacuoles. Leucocytes can often be detected in beginning their penetration of the uterine epithelium in Stage Two.

Stage Three.—In Stage Three, the stage of abundant cornified cells in the vaginal smear, vacuolar degeneration of the uterine epithelium is most typical (figs. 29, pl. VIII, and fig. 30, pl. V). Thus it is possible to say that the time when epithelial dehiscence and degeneration are most expressed in the vagina is the time also of epithelial impairment in the uterus. As we shall establish farther on, this degeneration can be detected in its incipiency before the occurrence of ovulation, that is, in the time interval between copulation and ovulation. In no cases have we seen degeneration proceed so far as to cause a denudation of the uterus.

Stage Four.—In Stage Four, characterized by the abundance of leucocytes which have extensively infiltrated the vaginal mucosa and have now reached the vaginal lumen, leucocytic infiltration of the uterus is also very abundant. Vacuolar degeneration reaches its maximum expression in this stage (fig. 36, pl. V). Epithelial regeneration and replacement in the uterus is apparently going on *pari passu* with the degeneration, and early in the dioestrous interval a perfectly normal, columnar structure is always found (fig. 9, pl. V).

From the foregoing it will be clear that the uterus participates in a marked degree in the active changes which characterize all portions of the reproductive system in the various phases of the oestrous cycle, that besides its peculiar secretory activity during the time of oestrus proper it undergoes to some extent the same wave of epithelial degeneration and leucocytic invasion that characterizes the vagina. It is doubtful whether these are analogous to menstruation, but in the rat, if we were to judge from the time relations alone, we should have no hesitation in deciding between the contrasting theories for the significance of menstruation according to which the uterine changes are considered either as a "freshening" or preparation, for implantation, or as an abortion due to failure of the latter. The chronology of events in the rat would lead us to adopt the first of these two views, for epithelial impairment is not only far advanced by the advent of ovulation but regeneration is complete before the eggs can reach the uterus. The tubal journey in the rat consumes three days. As a matter of fact, even in other forms where uterine degeneration follows rather than precedes ovulation, the egg may consume a time interval in the tubal passage sufficient for both uterine epithelial decay and reëstablishment. It will probably prove to be the case that in all the Mammalia ovulation delivers eggs into a healthy uterine epithelium. In the Eutheria this epithelium has been freshly regenerated; in the Marsupials it is somewhat older.

C. Changes in the Oviduct

There is some evidence that the oviduct also participates by structural changes in the events characterizing the oestrous cycle. We shall, however, at this time mention only the most marked and macroscopically detectable change in it, a change which is correlated with its functional capacity for receiving and conducting the egg toward the uterus immediately after ovulation. Although this somewhat anticipates the remarks we shall make under the section devoted to the ovary and ovulation, we may state at once that, in contrast to the condition before ovulation (figs. 38, p. 27, and 40, pl. I), for a period of at least approximately twelve hours after the estimated time of ovulation, the distal folds of the oviduct are distended with fluid (fig. 39, p. 28). These fluid-containing, distal loops of the oviduct also always contain the eggs, the presence of which

may be detected in the gross under the binocular microscope whether in the living animal or when the organs are placed in Locke's or Ringer's solution, by the movable, opaque mass seen through the rather transparent tubal walls. Sections (fig. 41, pl. I) show that the masses in question consist of the eggs surrounded by granulosa cells. In the large majority of normal cases, eggs will thus be found in the fluid-filled, distal fold of the oviduct at the beginning of Stage Four, characterized by the first appearance of leucocytes among the cornified cells in the vaginal smear. As the egg enters the intermediate and proximal portions of the oviduct, a similar distention by fluid does not accompany it.

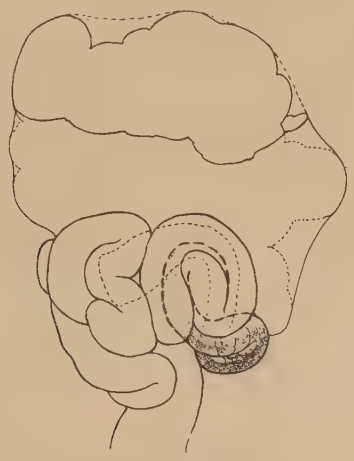


Fig. 38. The oviduct at any time when eggs are not present in the distal portion, which is stippled. The part shown by dotted lines is the ciliated "fimbriated" end. The periovarial membrane clings closely to the ovary. × ca. 10.

D. Changes in the Ovary

1. OVULATION

There can be no doubt of the coördination of ovarian changes with those already described for the vagina and uterus. All that series of changes which culminates in ovulation and the production of corpora lutea has a very definite relation to the various phases of the oestrous cycle as exhibited by the vaginal smear. The exact time relations which ovulation exhibits both with respect to the vaginal epithelial dehiscence and to copulation are, of course, of peculiar importance, for this may have a bearing both on the theories for the significance of menstruation and the fact of occasional unequal embryonic development in pregnancies of the same copulation age.

The structure of the ovary of the rat at the height of its sexual life and when isolated from males for two or three months is so complex that it would almost seem to forbid analysis. This is due not only to the fact that ovulation, and, consequently, corpora lutea occur at every one of the rapidly succeeding oestrous cycles of four or five days, but also to a remarkable persistence of the corpora, so that many "sets" of them are found in the ovary at any one time, sets which

Fig. 39. The oviduct soon after ovulation when eggs are present in the large distal fold shown at the right. The corresponding parts in figures 38 and 39 are stippled. The periovarial membrane is also distended with fluid and separated from the ovary. The "fimbriated" end is in dotted outline.

exhibit such slight variations in degree of regression as determined by their structure that it is practically impossible, without some way of marking them, for us to gain any sound notion of their age. It is not unusual to encounter more than fifty of these structures in a satisfactory state of preservation in a single ovary. In addition to this multiplicity of corpora lutea, one finds also in the rat's ovary a varying amount of follicular atresia, with which the formation of interstitial tissue is well known to be connected. On the other hand,

the sizes and structure of the normal follicles, though not suffering such great variation, as a matter of fact are related clearly to the oestrous cycle only when marked growth changes take place, so that it might be equally difficult to predict from the follicles with what phase of the oestrous cycle we have to do.

The above remarks will serve to indicate that the structure of the ovary does not show marked differences during most of the period occupied by the succession of normal ovulation cycles, but, as we will show in a moment, this is due to the fact that the outspoken changes identified with ovulation occur only for a brief time preceding and succeeding this event. In other words, the growth and rupture of the follicles and the formation of new corpora lutea are phenomena which do not occupy any considerable span of time. This fact emphasizes again the ease with which ovulation could be entirely overlooked did one not have the opportunity of examining the ovary with exact reference to the epithelial changes in the vagina.

In order to depict the ovarian changes that can be associated with the oestrous cycle, we may begin with the period in which the follicles are smallest; this is the period immediately succeeding an ovulation. We may state at once that ovulation occurs during the last hours of the cornified cell stage (Stages Two and Three), and has always taken place by the end of that stage, that is, when leucocytes first appear in the vaginal smear. We have previously stated this fact in another way under the caption "Changes in the Oviduct" when we said that at the end of Stage Three one may always find eggs from a recent ovulation in the distal folds of the oviduct. At the same time "new" corpora lutea of ovulation are present in the ovary.

With the approach of oestrus and during Stage Two, with the consequent hyperaemia of the entire sexual apparatus, growth of certain of the follicles becomes more marked, a process which slowly continues until the more rapid ripening changes take place late in Stage Two.

After Stage Three is clearly expressed, the stage in which considerable macroscopic cheesy accumulations of cornified cells occur within the vagina, the steps introductory to maturation may be detected. These consist in beginning indipping of the theca interna folliculi and its capillaries so that the membrana propria of the granulosa is sharply indented at these points. Corner has called attention to this in the sow. The egg as yet is still in the germinative vesicle stage; and the total dimension of the follicle does not exceed eight-tenths of a millimeter (table 2, p. 31). In our experience these changes take place about eighteen hours after the first appearance of cornified cells in the vaginal smear, at the time consequently when the majority of animals are no longer in heat. At some time during the next few hours, although the follicles do not increase appreciably in size, the cumulus displays a distinct corona radiata and the first maturation spindle is formed. The slight thecal indippings noted above may be

somewhat more marked. There then takes place a final swelling of the Graafian follicles, so considerable that they may attain diameters of nine-tenths of a millimeter. At this time the infoldings of the theca folliculi have become very conspicuous (figs. 42 and 43, pl. XI) and some of the outermost granulosa cells have minute lipoid deposits within them. Now the first polar body may be seen, the second maturation spindle being in place; and in this form the ovum is shed to begin its journey in the tube.

While our data indicate that the above statements represent the average condition, they also disclose a considerable variation in the exact time of incidence of any of these phenomena. It is true, as we have indicated, that in most animals ripening changes begin about the eighteenth hour after the first appearance of cornified cells in the vaginal smear and that ovulation may be expected at the twenty-fourth hour dating from the same event. However, ovulation may occur at any time in the twelve-hour interval embraced from the eighteenth to the thirtieth hour after Stage One. As regards the exact time involved in the various steps of ripening, we have not been able to establish a reliable opinion. For this it would be necessary, in view of the variation which exists, to accumulate a very large body of data. It is our impression that the remainder of the ripening changes take place very rapidly after the first maturation spindle has once been established. The sequence of events in the ripening process which we have been able to discover has some value inasmuch as various criteria have been advanced from time to time from the work of Bischoff onward which would enable us to recognize a fully mature ovarian egg. But it is possible that the exact sequence of events which we have described for the rat will not have general application. To attempt a statement of most general application, it would appear that it is not, indeed, until the formation of the first polar body that we may state that the Graafian follicle has nearly reached the time of rupture.

Our records of the histological findings in about seventy-five animals form the basis of the above account. Thirty-seven of these animals were sacrificed solely with reference to this question, being killed at intervals of twelve, eighteen, twenty-four, thirty-three, and thirty-six hours after the incidence of cornified cells in the vaginal smear. Exact findings are detailed in table 2, page 31.

Many of the cases in this table permit us to see the changes which are immediately consequent upon ovulation, and, in particular, the inception of the corpus luteum. The young corpora have very large cavities within them (figs. 41, pl. I, and 44, pl. XI). The closure of the pore of rupture must be very rapid since we have failed to detect it in this relatively large series of cases. Somewhat later stages in the formation of the corpus are seen in the cases of animals 3827 and 4093, where the uninvaded central space is smaller.

TABLE 2.

Summary of findings in rats killed for the determination of the time of occurrence of ovulation.
(Not included in Table 30)

Rat	Stage	Killed hours after first cornified cells	Diameter of follicles in millimeters	Inpushings of follicle wall by bloodvessels	Egg	Oestrus
4007	1	0	.65, .70	Some	Germinative vesicle	
4326	2-3	18	.68, .70	None	Germinative vesicle	Not yet in heat
3954	2-3	18+	.65, .68, .70, .70	Beginning	Germinative vesicle	Past
4058*	2-3	18	.80, .83	Present	Germinative vesicle	
4099*	3	18	.78	Beginning	Germinative vesicle	
4237	2-3	24	.65, .70, .75	Several	Germinative vesicle	
4060	2-3	27+	.80, .85	Some	Germinative vesicle	First time when killed
3994	2-3	36	.70, .75	Beginning	Germinative vesicle	
4009	2-3	36		Some	Germinative vesicle	Still in heat
4001	?	162	.75, .75, .80, .80	Marked!	Germinative vesicle	Still in heat. Never free of leucocytes.
3824*	2-3	18	.70, .70, .80	Slight	Germinative vesicle and first spindle	
3815	2-3	15	.75	Present	First spindle	
4065	2-3	24	.70, .80	Slight	First spindle	
4081*	2-3	24	.65, .75, .75, .90	Some	First spindle	In heat
4234	2-3	24	.70, .70, .70, .73, .80	Some	First spindle	
4027	2-3	30	.70, .70, .75, .80	?	First spindle	In heat
4036	2-3	27	.75, .80, .85	Present	First polar cell	Still in heat
3784*	2?	30	.80, .80, .88, .90, .90	Large	First polar cell	
				Corpora lutea		
4078*	2-3	18		Large cavity	In distal fold of oviduct.	
4102*	3	18		Large cavity	In distal fold of oviduct.	
4070	2-3	21		Large cavity	In distal fold of oviduct.	Past
4238	2-3	21		Large cavity	In distal fold of oviduct.	
4095*	3	24		Large cavity	In distal fold of oviduct.	Past
4052*	2-3	24		Large cavity	In distal fold of oviduct	Past
4056*	2-3	24		Large cavity	In distal fold of oviduct	
3799*	3	30		Large cavity	In distal fold of oviduct	Past
4008	2-3	30		Large cavity	In distal fold of oviduct	
4128*	3	30		Large cavity	In distal fold of oviduct	
4239	2-3	33			In distal fold of oviduct	Still in heat
4033	2-3	33		Small cavity	In distal fold of oviduct	Past
4126*	3	36			In distal fold of oviduct	
4038	4	114			In distal fold of oviduct	Past
3827	4	84		Small cavity	Near intermediate portion	Past
4047*	2-3	30		Small cavity	Entering intermediate portion	Past
4093*	2-3	18		Small cavity	In intermediate portion	
4066	2-3	30-33			Near intermediate portion	Still in heat

*Observations started in Stage 1 and samples taken carefully to avoid any possible unfavorable effect on cycle.

Another feature characteristic of the period of ovulation never before to our knowledge mentioned in the literature is the accumulation of fluid in the periovarial space, causing a distention of the periovarial membrane (fig. 39, p. 28), which, in pathological cases, may be very great. As will presently be shown, the distal portion of the oviduct also becomes distended with fluid. The fluid in both is probably directly secreted into the cavity in question and is not derived from the follicles, for the fluid in the latter behaves differently in that it alone in fixed preparations produces with the reagents a granular precipitate.

The migration of the ovum can be followed with some accuracy. As we have just indicated, in the rat the first polar body is formed by an ovarian egg, as has been demonstrated to be the case for the mouse, guinea pig, rabbit, cat, sow, bat, armadillo, opossum, and Dasyurus. The second maturation spindle for the second polar body must form very soon after the first, for it is in place in the ovarian egg just before rupture, but the second polar body appears not to actually form in unfertilized eggs. During the first twelve hours after ovulation, the egg with the second maturation spindle in position moves slowly through the first two or three distended distal loops of the oviduct. During the next twenty-four hours it traverses the intermediate part of the proximal loops, and during the third day it will be found at various positions in the proximal loops, where, in case it has not been fertilized, it degenerates. This degeneration consists in a characteristic, unequal, direct fragmentation, or segmentation, of the protoplasmic mass which may or may not carry with it unequal sized nuclei, as shown by some unpublished observations by Margaret Mann. One may see a similar form of degeneration of the ovarian egg in the case of follicular atresia, where also one may have a rather orderly imitation of blastogenesis, or parthenogenesis, as L. Loeb has called it. The same thing may also occur in the case of an ovum retained in a corpus (fig. 45, pl. XI).

2. RELATION BETWEEN OESTRUS AND OVULATION

The relation between oestrus and ovulation has a very special interest from the standpoint of the time of occurrence of fertilization. We therefore discuss it here and begin with a more accurate description of the time of appearance of oestrus and the variations in that time.

Usually oestrus is manifested for a period of from nine to twelve hours, beginning in the last part of Stage One and occupying most of Stage Two. Instances of the long duration of oestrus, of its early occurrence, or of its late occurrence are not unusual; nor must we fail to note another peculiarity, that is, the occasional occurrence of the manifestation of oestrus for only three hours or a similar very short interval. We have accumulated these results by the method already indicated, i.e., by offering individual females to a cage of males at the three-hour intervals when a record was kept of the vaginal smears.

Early and late exhibitions of oestrus have a special significance when brought into relation with ovulation. They might enable us to explain the variation found in the development of young embryos when the only age criterion is the copulation date. Table 2 indicates that ovulation may occur as early as eighteen and as late as thirty hours after the first appearance of cornified cells in the vaginal smear. Oestrus may be exhibited as early as three hours before the appearance of cornified cells or as late as twenty hours after. Did ovulation succeed oestrus by an invariable interval of time, the phenomenon of unequally developed embryos at the same copulation age would have to be explained by the unequal rate of ascent by the spermatozoa through the genital passages. This explanation suffers, however, first, from the belief in the limited viability of unfertilized eggs, and, secondly, from the fact that the enormous number of sperm ejaculated make it likely that the eggs are reached in an approximately uniform time.

The last objection gains weight in the case of those forms which, like the rat, have ovulation normally at a very considerable time interval after copulation. In the case of the rat almost a whole day separates these events. Long has demonstrated that sperm may reach the distal part of the oviduct in the mouse, a related form, within four hours. In view of all the facts, then, it is extremely probable that, in the rat, sperm will usually await the arrival of the ovum for a considerable interval of time, i.e., fertilization ensues immediately upon ovulation. Now it is very likely that ovulation does not necessarily follow oestrus in a uniform period of time, for there is reason for relating it to the general progress of the oestrous changes taken as a whole, an indication of the orderly progression of which we have found in the vaginal smear. In the case of very late exhibition of oestrus associated with precocious ovulation there might, then, be an actual transposition of these two events and a failure of fertilization through the inability of ova to continue to maintain their vitality until reached by sperm. It is evident that the cases of speediest fertilization following copulation will occur under conditions in which oestrus and ovulation are nearest together, providing sufficient time (at least four hours) elapses for the sperm to accomplish the uterine and tubal journey; and, conversely, the greatest delay in fertilization would occur in those instances in which oestrus and ovulation are separated as far as possible from each other. For example, if oestrus is manifested three hours before the occurrence of Stage One and ovulation thirty hours subsequent thereto, the sperm would have to wait thirty-three hours before fertilizing the eggs. Thus age differences in embryos dated from the hour of copulation could easily arise and could conceivably involve somewhat over a day.

3. THE POST-PARTUM OVULATION

In the rat, as is known in other animals, an ovulation occurs shortly after parturition. The post-partum ovulation may take place at any time between the sixteenth and twenty-fourth hours after littering, as indicated in table 3.

TABLE 3.

Showing number of hours after parturition when eggs were procured from the oviduct.

Hours post partum	No. of instances when eggs were found	No. of instances when eggs were not found
15		1
16	1	
17	3	1
18	5	1
19	5	2
20	3	
21	5	2
22	5	5
23	2	
24	11	6
25	7	
26	4	
27	1	
28	2	
29	3	
30	3	
32	3	
33	2	
36	1	
37	1	
43	1	
44	1	
	69	18

4. CORPORA LUTEA

Whatever may be the exact mechanism of follicular rupture, we must regard this as an influence widely distributed in the ovary as a whole, for ovulation is characterized by the practically simultaneous rupture of all the follicles belonging to that particular "set." In the examination of scores of complete series of ovaries at times just preceding and subsequent to ovulation it is remarkable how seldom one encounters instances of a lack of synchronism in this phenomenon. At the same time that we make these statements, we would call attention to the occasional apparent failure of a single, or, at most, of two follicles to undergo rupture along with their mates, and to the occurrence of follicular atresia in such large follicles, but whether or not the atretic process has begun before ovulation we are unable to state.

Corpora lutea atretica sensu strictu.

We have also encountered a rarer condition: a highly peculiar type of atresia in those occasional follicles where there has apparently been no rupture. In the cases to which we refer the granulosa does not undergo the degeneration characteristic of atresia but proceeds to the formation of lutein cells similar in all respects to those of the fresh corpora produced from the other ruptured follicles of this set, but surrounded by theca cells which remain in place and are transformed into interstitial tissue.

Corpora lutea with retained ova.

Finally, there is the somewhat commoner process, namely, the production of typical corpora lutea by follicles in which the eggs have still been retained *in situ* (figs. 45, pl. XI, and 59, pl. VI). In such instances the thecal cells behave in no way different from their rôle in the formation of normal corpora lutea. They do not produce interstitial tissue, but to an extent not yet determined they participate in the ingrowth of connective tissue which accompanies the blood vessels of the corpus. In other words, a typical corpus luteum is formed, in the center of which, however, the egg is found. This phenomenon is by no means unknown, but we doubt whether the frequency of its occurrence has been suspected by any previous investigator. In order to obtain more data upon this point, we undertook to examine carefully all those cases in which the number of eggs discharged at an ovulation could be ascertained with certainty as well as the number of corpora lutea from that particular ovulation (table 4). In these cases, ova in a good state of preservation were encountered in the distal portion of the oviduct and their number could be counted with reliability. Thirty-seven such cases were found suitable for our purposes. In each case we studied only a single oviduct and ovary. In twenty-five of them the number of ova encountered corresponded strictly with the number of fresh corpora found in the corresponding ovary, and in twelve cases the number of corpora was in excess of the number of eggs found in the oviduct. In some of the latter instances, moreover, it was possible to discover that the ova which were missing in the oviduct were at the center of certain of the corpora; but it is to be pointed out that a rather rapid degeneration of these retained ova takes place, so that in our opinion in many instances where they previously existed they were not encountered by us.

It would appear, then, that about one-third of all ovulations exhibit one or more instances of retention of the ovum within the corpus luteum, but the total number of normal and abnormal corpora which we have thus assumed to exist in these thirty-seven ovulations stand in the ratio of nine to one. Since, in our experience, about ten follicles mature at each ovulation (table 4), the above statement might imply that one of the corpora in each ovulation is apt to have

a retained ovum, but the above analysis would indicate that only about one-third of all ovulations show such abnormalities and that the nine to one proportion of typical to atypical corpora is due to the multiple occurrence of abnormal corpora in the same ovulation.

TABLE 4.

Table showing correspondence between the number of eggs in the oviduct and the number of corpora formed at that ovulation (in one ovary and oviduct).

A	Average number of corpora lutea in 54 cases	=	5.37	in one ovary
	Average number of eggs in 50 cases	=	4.8	in one ovary
B	Average number of corpora lutea in 37 selected cases in which both corpora and eggs in oviduct could be counted	=	5.4	in one ovary
	Average number of eggs in same 37 cases	=	4.8	in one ovary

Average number of corpora lutea produced at each ovulation.

It would appear that on the average five corpora lutea are produced in each ovary at any one ovulation. One or both ovaries from one hundred and eighty-two ovulations have been analyzed in order to reach this conclusion. The averages obtained are exhibited in the subjoined table.

TABLE 5.

Table showing number of corpora per ovulation in cases in which the determination is certain.

95 pregnant animals	Average 4.93 in one ovary
28 nursing animals	Average 5.7 in one ovary
40 animals—corpora lutea of first ovulation	Average 5.45 in one ovary
19 animals—corpora lutea of second ovulation	Average 5.05 in one ovary
	General average 5.20 in one ovary

Although five is thus the average number of corpora per ovulation in a single ovary, as many as ten corpora and as few as one have been encountered. The distribution of instances of the number of corpora lutea produced at a single ovulation may be seen from table 5*a*, which is merely a re-arrangement of the data from which table 5 has been secured, together with the addition of seven new cases.

Size of corpora lutea of ovulation.

The corpora lutea of ovulation when fully formed usually measure about 1 millimeter in diameter. The maximum measurements which we have recorded are of diameters of 1.2 millimeters. Succession of the corpora by another set is not immediately indicated by size regression of the first set, whose dimensions are indeed not perceptibly reduced even by the second succeeding ovulation when their greatest diameter is still about a millimeter. As far as we have been able to determine, the corpora lutea of ovulation do not differ from those of pregnancy or pseudopregnancy except in the larger size which the latter type of corpora usually attain. The corpora of pregnancy, however, do not exceed those of ovulation until after the tenth day of gestation.

TABLE 5a

Table showing the number of instances in which various numbers of corpora lutea were produced in a single ovulation.

Number of corpora lutea produced at a single ovulation	Instances
1	3
2	8
3	21
4	42
5	41
6	29
7	26
8	4
9	2
10	3

Functional life span of corpora lutea ovulationis.

Two points of view make it important for us to attempt to determine with some precision the functional life span of the corpora lutea of ovulation. We refer to the exact relation of the corpora lutea to the oestrous cycle and to the confusion created by the retention of corpora lutea in the rat for many oestrous cycles succeeding their formation. It is obvious from the great number of corpora which occur in rat ovaries that any outspoken degeneration of the corpora lutea of any particular ovulation can not occur before the next oestrus brings on the succeeding set. But since in other forms corpora lutea, as long as they are functionally active, are supposed in some way to prevent ovulations and to hold off the oestral degenerative changes which characterize the mucosa of the reproductive system, it was important for us to determine whether or not the conditions in the rat invalidate this assumption. In other words, it was important to detect, if possible, any morphological changes which would indicate a

diminution or change in the function of the corpora lutea of ovulation just preceding the next oestrus. We have found these morphological changes in size and distribution of the fats which brown with osmic acid, and interpret this change in the amount, or, more properly, aggregation, of the lutein cell fat to be the expression of a change in the physiology of the cell. Our assumption seems the more likely inasmuch as exactly parallel changes occur in the corpora lutea of ovulation, of copulation or pseudopregnancy, of gestation, and of lactation, as we will show later on; and in the latter cases these changes are definitely related to the cessation of the gravid or of the lactating state.

In the ripe Graafian follicle just preceding rupture one may observe that the cells of the theca interna are well laden with fair sized lipoid spherules which blacken readily with osmic acid, whereas the granulosa cells, later to become the lutein elements, have no appreciable fat, or, at most, minute brown granules, in their outermost layers next the basement membrane which separates them from the theca interna. Very young corpora have the same minute granules (fig. 47, pl. VII). Thirty hours after the rupture of the follicle, this difference in the fat content of the theca interna and granulosa has disappeared, and the enlarged granulosa elements, now the lutein cells, possess small lipoid droplets which brown in the osmium tetroxide and have the dimensions and distribution shown in figure 48, plate VII. In order to obtain uniformity in measurement, the granules were measured by means of a special eyepiece. In this ocular was placed a disc of glass on which had been sprayed exceedingly minute particles of India ink, all of which were then removed except nine, which were selected to form a graded series. The lipoid granules in the preparation were easily compared with these spots in the eyepiece and designated according to the number of the spot or spots with which they corresponded. The spots are shown in figure 46 as they would appear in a drawing at 750 diameters.

Fig. 46. The spots on the ocular micrometer disc and 10 divisions of a millimeter divided into hundredths drawn with the same objective (Zeiss 2 mm. apochromat) and ocular (6 compensating) used in measuring lipoid granules in lutein cells. The spots are numbered from 1 (smallest) to 9 (largest). Larger granules are measured in multiples of 9 (i.e., 3×9). $\times$ 750. Same magnification in figures of lipoids, plate VII.

The lutein lipoid droplets are highly refractive when viewed in a drop of Ringer's solution in which the fresh lutein cells have been crushed by a cover glass; when fixed with formalin, they are stained red by the application of a saturated aqueous solution of Nile Blue Sulphate to the frozen sections, which are then rinsed in distilled water and transferred to a 1 per cent sodium hydroxide solution. We have found that the best way of fixing and preserving them is by the employment of Meves' modification of Benda's Fluid (Meves and Duesberg, 1908), and by the after treatment of such fixed material with pyroligneous and chromic acid and potassium bichromate.

The ovary is transferred from Benda's fluid after 48 hours, rinsed in distilled water, put into a solution of equal parts of 1 per cent chromic acid and pyroligneous acid for 48 hours, rinsed again in distilled water, and is then transferred to 2 per cent aqueous bichromate of potassium for 48 hours. After this it is washed for from 12 to 24 hours in distilled water and dehydrated, cleared for from 2 to 4 hours in cedar oil, transferred to xylol and imbedded in paraffine.

The tissue may be sectioned at any thickness and the sections mounted on slides; the lipoid may then be examined directly with the oil immersion lens, the oil serving to make the paraffine sufficiently transparent. Without the treatment with pyroligneous and chromic acid and potassium bichromate, the lipoid granules would dissolve easily in the xylol and balsam. Even after this treatment, they are sufficiently soluble in balsam that preparations a year old are somewhat reduced in intensity when compared with uncovered paraffine sections.

These granules are small and strikingly uniform in size, especially in the same cell, although they are somewhat larger than the extremely minute fat droplets which we have mentioned as appearing in the granules at about the time of rupture, and are larger than the very small lipoid granules which, we shall show later on, are characteristic of the cells of the corpus luteum of lactation. They are nevertheless very much smaller than the granules of degenerating corpora, which, moreover, never have the striking uniformity in size. As a rule, they are also uniformly distributed in the cell and even in the cases where that distribution is uneven they are not so crowded together as to be in contact.

At the time of onset of the next oestrus some of these lipoid deposits in the corpus are appreciably larger in size and their number is increased (fig. 49, pl. VII). These changes occur first in the inner hemisphere. It is this morphological change that, in our conviction, is correlated with a functional impairment of the lutein cells. The lipoid changes, of course, progress farther; they attain a maximum and regress before the corpus actually begins to diminish in size or to undergo what would ordinarily be called outspoken degeneration. It is important to know that the lipoid changes are very marked before any other degeneration of the lutein elements or diminution in the size of the corpus can be detected. We have already noted that a diminution in size of the corpus does not usually take place until the beginning of the third cycle succeeding a corpus of ovulation. Early in the succeeding oestrous cycle the sizes of the lipoid

granules continue to be even more clearly unequal and the granules are more irregularly distributed in the cell, while their number is greatly increased. This appearance of larger lipoid granules may continue until the cell may contain one or more bodies considerably larger than the nucleus, but succeeding this phase the lipoid decreases.

At the beginning of the third cycle the lipoid becomes much reduced in amount; and it is when this phase of diminution occurs that there is at last perhaps a slight reduction in the size of the corpus as a whole. By the time that the lipoid content of the lutein cells is notably reduced, macrophages are clearly abundant in the corpus and are gorged with fatty substances which, in spite of the after treatment with pyroligneous acid, etc., is never black, but brownish; whereas the Nile Blue Sulphate method shows that some of these great phagocytes have fatty acids within their vacuoles, substances never present in the lutein cells and indicating a possible splitting of the ingested lutein cell fat.

Ovaries fixed with osmic acid at any time during the succession of normal ovulation cycles thus show sets of corpora lutea which vary in the size, number, and distribution of their lipoid bodies in accordance with their age in the way which we have just described. (Consult also pl. VII, and figs. 72, 83, 51, pl. VIII; 53, 89, pl. IX; 73, 54, 52, 82, pl. X.)

We may summarize our views by stating that our studies on the morphology of its lipoids indicate that the corpus luteum cell has reached its greatest functional activity at the end of the oestrous cycle which gave it origin, and that at the beginning of the next oestrus it is in a state of regression. Accordingly, any corpus that contains large lipoid granules is one which is already degenerating. The considerable difficulty in deciding in the case of the corpora lutea of ovulation whether degeneration precedes or follows the exact time of incidence of the next oestrus has proved easier to surmount in the case of the corpora of gestation and lactation, where, as we will show later, degenerative lutein changes are separated by a somewhat longer time interval from the next oestrus.

The reader may naturally inquire how it is that we have been emboldened to identify, in the complex ovary of the rat, any particular set of corpora lutea of ovulation with a known ovulation; and, furthermore, how we knew we were dealing with the corpora of ovulation toward the end of of an ovulation cycle, inasmuch as these cycles vary in length. It is consequently necessary to explain how we oriented ourselves with certainty in this matter.

Lactating mothers were treated with intraperitoneal doses of the vital dye, Dianil Blue 2 R (see p. 60), in order to mark with unmistakable blue deposits the lutein cells of the corpora of lactation and of the preceding pregnancy. The young were then removed and the first spontaneous oestrus, as determined by the vaginal smear, noted, when the corpora of that ovulation were examined by extirpating one of the ovaries. Possession of the other ovary enabled the animal in most instances to exhibit the next oestrus at a normal time (i.e., four to five days later), and in such cases we could be certain that we were dealing, in the case of the extirpated ovary, with the condition of the corpus at particular times in dioestrous intervals of normal length. A fuller explanation of the matter may be afforded by recounting a typical protocol from many such which were employed in our work. (See description of fig. 53, pl. IX.)

Instances can be found of the existence of from fifty to seventy-five corpora lutea in a single ovary of the rat, though many of these are badly degenerated and some diminutive. In all cases the last four or five sets (i.e., from twenty to thirty corpora) are substantial, fairly well preserved structures, a fact which tallies well with our counts of the number of well preserved corpora that are encountered about twenty days postpartum. An analysis of many of the latter cases gave us an average of thirty corpora lutea at twenty days post-partum, so that when five of these are allowed as corpora of pregnancy and five as corpora of the post-partum ovulation, twenty are left to represent, probably, four ovulations of five corpora each at five-day intervals. No such persistence of the corpora lutea of ovulation takes place during the gravid condition, for on the twentieth day of pregnancy, instead of encountering thirty corpora in each ovary, as occurs twenty days post-partum, we encounter usually merely the five large corpora lutea of gestation, which have thus not merely inhibited ovulation, but have also brought to decay and complete resolution all other corpora occurring in the organ.

We were able to make the above observations by taking advantage of the fact that at the end of pregnancy practically no corpora of ovulation are visible. By permitting the corpora of the spontaneous ovulation on the day of littering to occur and by breeding the animal at the incidence of the next oestrus, we secured the conditions required, that is, we were able to say that the only corpora of ovulation present toward the end of the second gestation were those of the first post-partum ovulation which intervened between the two pregnancies. But in order to still further distinguish these with certainty from the corpora of the first pregnancy, we administered a vital dye to the animal from the sixteenth to the twentieth day of the first gestation, thus marking clearly the corpora of the first gestation. There were thus three kinds or sets of corpora in these ovaries and only three—the corpora of the first pregnancy which were blue, those of the post-partum ovulation, badly degenerated, and those of the second pregnancy.

E. Correlation of Changes in the Vagina, Uterus, Oviduct, and Ovary

The correlation of the changes which we have thus demonstrated to take place during each one of the stages of the oestrous cycle in the various portions of the reproductive system of the rat is best summarized and visualized in table 1.

F. Length of the Various Portions of the Oestrous Cycle

In order to have a correct conception of the length of the various portions into which, by changes in the vaginal smear, we have divided the oestrous cycle, it was necessary to make observations on a relatively large group of animals separated by the shortest practicable time interval. After some preliminary work with especial reference to the number of animals which had to be handled, we settled upon the proper interval between examinations as one of the duration of three hours and preliminary to this work made for some weeks a daily examination of several hundred animals in order that we might employ for the

final work those with regularly recurring four to five day cycles. About one hundred animals were thus selected for this work, but the abnormalities introduced by this handling did not permit more than about sixty of them to complete their oestrous cycles in a time to be regarded as practically normal and hence available for this purpose. In many cases the observations were carried well into a second cycle so that in the same animal two or more instances of any one portion of the cycle were secured. Our results are best summarized in tabular form.

TABLE 1.

Schematic outline of changes in the reproductive organs of the rat during the œstrous cycle.

Stage	Living Animal	Histology of vaginal mucosa	Uterus	Ovary and Oviduct
1 (12 hrs.)	Vaginal mucosa slightly dry. Smear of epithelial cells only. Lips a little swollen. In heat toward end.	Many layered (8-12) .08-.1 mm. thick. Mitoses active. Cornified layer *under* superficial layer of epithelium. No leucocytes.	During Stage 1 uterus becomes distended with fluid increasing in diameter from 2.3 to 3.7 mm.	Follicles large
2	Vaginal mucosa dry and lusterless. Smear of cornified cells only. Lips swollen. In heat.	7-11 layers of cells .08-.1 mm. thick. Cornified layer well formed and superficial. No leucocytes. Mitoses fewer.	Reaches greatest distention (5 mm.) and thinness of epithelium and then regresses to diameter of 1.8 mm. Vacuolar degeneration sometimes begins.	Follicles largest. Eggs may undergo maturation.
3 (**2** and **3** 27 hrs.)	As in Stage 2, but cornified material abundant (cheesy) and animal not in heat.	5-9 cells thick. .064 mm. thick. Cornified layer loose and finally completely detached. No leucocytes. Mitoses still fewer.	Diameter of uterus about 2.0 mm. Epithelium undergoing vacuolar degeneration.	Ovulation. Secretion of fluid into periovarial space and oviduct.
4 (6 hrs.)	Vaginal mucosa slightly moist. Smear of cornified cells and leucocytes. Swelling of lips gone.	4-8 cells thick. .062 mm. thick. Cornified layer gone. Many leucocytes. Mitoses increasing.	Diameter of uterus 2.2 mm. Some vacuolar degeneration but also regeneration.	Young corpora lutea. Eggs in oviduct. Follicles smallest.
5 Dioestrous interval (57 hrs.)	Vaginal mucosa moist, glistening. Smear of leucocytes and epithelial cells. Variable amount of mucus.	4-7 cells thick. .042 mm. thick. Leucocytes. Mitoses not numerous.	Diameter 1.7 mm. Epithelium undergoing regeneration.	Follicles of various sizes Corpora lutea continue to grow. Eggs traversing oviduct throughout early interval.

TABLE 6.

Table showing the length in hours of Stage One of the œstrous cycle of the rat. (Vaginal smear consisting of abundant, uniform-sized, nucleated, epithelial cells only.)

Length of Stage 1 in hours	Number of instances
3	3
6	8
9	11
12	40
15	20
18	17
21	14
24	5
27	1
30	1

TABLE 7.

Table showing length in hours of Stages Two and Three of the œstrous cycle of the rat. (Vaginal smear consisting of cornified cells only.)

Length of combined Stages 2 and 3 in hours	Number of instances
12	2
15	0
18	3
21	3
24	7
27	14
30	6
33	13
36	6
39	7
42	9
45	8
48	6
51	3
54	4
57	2
60	2
63	3
66	0
69	2
72	0
75	1
78	0
81	1

TABLE 8.

Table showing length in hours of Stage Four in the œstrous cycle of the rat. (Vaginal smear consists of cornified cells and leucocytes.)

Length of Stage 4 in hours	Number of instances
3	23
6	40
9	19
12	13
15	7
18	4
21	0
24	0
27	1

TABLE 9.

Table showing length in hours of Stage Five, or the Dicestrous Interval, in the œstrous cycle of the rat. (Vaginal smear consists of leucocytes and nucleated epithelial cells.)

Length of Stage 5 in hours	Number of instances
30	2
33	1
36	1
39	1
42	6
45	3
48	10
51	4
54	6
57	8
60	8
63	5
66	7
69	3
72	3
75	5
78	1

Mode: 48 hours Average: 43 hours

TABLE 10. SUMMARY.

Table showing length in hours of the component parts of the œstrous cycle of the rat.

Stage	Mode	Average
One	12 hrs.	14.2 hrs.
Two and Three	27 hrs.	38 hrs.
Four	6 hrs.	7.8 hrs.
Five	48 hrs.	53 hrs.

G. TOTAL LENGTH OF OESTROUS CYCLE

Observations were made on somewhat over three hundred females from four to six months of age and accordingly in full sexual vigor, having been from the time of weaning isolated from males. These animals formed the basis for our determination of the oestrous cycle in the rat as lying between four and six days.

We believe that the data present the normal spontaneous ovulations in this animal. Influence of the male was obviated by isolation of the sexes. Moreover, since on several occasions a female was observed to try to act the part of the male, lest even females might possibly have some influence on one another, twelve animals were kept in solitary confinement for about a month, at the end of which time it was clear from the cycles that there was no difference between the ovulation periods of such animals and those of females allowed to live together in small numbers. Complete data on which our results have been based may be found in Table 37, appendix. The data are also summarized in Table 11. The range of variation is from three days to twenty-eight, two cases being recorded, even, of spontaneous oestrous cycles of thirty-nine days' duration. As will be clear from the data, we are not inclined to attribute much importance in an understanding of normal phenomena to any cycle in excess of eight days, for 92 per cent of even a miscellaneous lot of animals have cycles that fall within the eight-day period (average 4.8 days). So great, in fact, is the proportion of the combined number of instances in which cycles of four, five, and six days were observed that we do not hesitate to designate this time interval (average 4.6 days), which comprises 82 per cent of our actual observations as the normal interval for this animal.

TABLE 11.

Table of observed instances of œstrous cycles of various lengths.

Length of cycle in days	Number of instances				
3	65				
4	789		Average		
5	634	82%	age 4.6	92%	Average
6	233		days		4.8 days
7	69				
8	60				
9	30				
10	24				
11	16				
12	22				
13 and over	57				
	1999 General average 5.4 days				

The reader, however, will desire some explanation for the fairly frequent occurrence of longer cycles and without further work upon this subject we present several concrete suggestions. Our colony was fed upon the fluctuating ration constituted by table scraps, and we have observed a tendency to the prolongation of the oestrous cycle in all cases of undernutrition; so, also, some disturbance in the exact regularity of feeding hours and the excitement produced by frequent handlings has given evidence of a slight prolongation of the cycle. Furthermore, impairment of respiration, which is invariably produced by allowing animals to pile up together in large cages and which we believe may even occur where three or four animals are confined narrowly, has, in our experience, lengthened the oestrous cycle. We thus have in our hands an extremely sensitive index of the well-being of the young adult female rat, an index more adequate to portray a sound physiology than the appearance of bodily activity, a glossy coat, normal weight, or any other sign known to us. The shortest oestrous cycle which occurs with any regularity is that of four days, and this remains also the most frequently occurring length of cycle in all our work. We are even, in fact, inclined to regard it as the true normal cycle, explaining the high number of instances of the five-day cycle as due to slight, but constantly operating, inadequacies in the hygiene to which our colony, in spite of our care, was submitted, though it will remain for future work with a superior hygiene to substantiate this surmise. We give our data exactly as they were found in order that the material with which we have dealt may be fully known and may be compared with that of subsequent investigators.

It is only necessary, in conclusion, to note that in our experience but slightly more than half of a miscellaneous stock of animals give oestrous cycles of such unfailing regularity that a discrepancy of more than forty-eight hours in the length of any one of them did not occur, and that, furthermore, even in these instances cases of an inexplicably longer cycle interpolated in a long series of regular cycles occurred. *We are thus acquainted with the necessity not only for a very superior hygiene but also for an exact individual oestrous history of every animal upon which reliable data as to the experimental physiology of the sexual system are desired.* In the material which this monograph presents no conclusions have been drawn except on the basis of the study of animals which we have submitted to this exact control.

H. Comparison of the Oestrous Cycle in Rat and Guinea Pig

The two forms in which the oestrous cycle is best known are the guinea pig and the rat. In making a brief comparison between them it may be said at the beginning that the correspondence in general between the two forms is close. After observing about twenty-two guinea pigs for several months, we feel that the correspondence may be even closer than indicated by the published descriptions of Stockard and Papanicolaou. In the first communications on the oestrous cycle in the rat (Long, 1919; Long and Evans, 1920) four stages were described. They were numbered from 0 to 3, instead of 1 to 4, solely for the purpose of making clear the homologies with the guinea pig, for it was believed that in the rat Stage 0 was an earlier stage than Stage 1 in the guinea pig. Furthermore, the rat has no stage corresponding to Stage 4 of the guinea pig. In other words, it seemed as though the oestrous changes in the rat began and ceased earlier than in the guinea pig. In their latest paper (1919) Stockard and Papanicolaou have divided their Stage One into two periods, thus making the first period of Stage One correspond to our old Stage 0 and the second period to our old Stage One. In this paper the stages are renumbered One to Four, instead of 0 to Three, since we believe that we have established an accurate foundation or standard for both rodents and one which we hope might serve for other forms of mammals. To make the guinea pig conform and also simplify it we would suggest that the two periods of Stage One be called Stages One and Two. and the former Stages Two to Four be designated Stages Three to Five. The older and the new terminologies of both rat and guinea pig would then be indicated in the following table:

Numerical designation of stages in oestrous cycle of rat and guinea pig.

Rat Old	Rat New	Guinea pig New	Guinea pig Old
0	1	1	1st period of Stage 1
1	2	2	2d period of Stage 1
2	3	3	2
3	4	4	3
		5	4

In the following comparison between the rat and the guinea pig the stages will be designated as in the two middle columns of the above table.

Stage One (*first period of old Stage One of guinea pig*).—In both animals there is a dry condition of the vaginal mucosa not very evident in either form. In the smear there are no leucocytes, but only squamous epithelial cells. In the rat these epithelial cells are somewhat different from those of the interval in that they are rounder and contain small granules or vacuole-like bodies which are much clearer in fresh preparations than in stained. In the guinea pig we have also been able to recognize these distinctive characteristics, so that with experience one can distinguish between these epithelial cells and those of the interval or of Stage Three of the guinea pig. A condition in the rat not yet described in any other mammal is to be found in the secretion of fluid into the uterus, which causes great distention of the latter. This accumulation of fluid reaches its maximum at the end of the stage and into the next stage (Two), when it disappears probably by escape through the vagina. It may be that this fluid corresponds to the mucous secretion in the guinea pig, which, as pointed out in the next paragraph, also escapes into the vagina. Copulation may occur late in this stage in the rat, not at all in the guinea pig.

Stage Two (*second period of old Stage One of guinea pig*).—In the rat the vaginal mucosa is conspicuously dry, having a strong, superficial cornified layer from which a few elements become detached to form the typical cornified cells of the smear. This is the usual time of copulation in both forms. In the guinea pig Stockard and Papanicolaou (1917) describe a transition to the next stage (old Stage Two) marked by the presence of a few of these cornified cells. We have found them in the guinea pig in as large numbers as in the rat, the appearance being almost identical, coming after the superficial epithelial cells. Moreover, in a few instances the vagina was dry at first, to become flooded with mucous almost immediately. We believe that Stockard and Papanicolaou have underestimated the importance of this condition, representing as it does the cornified layer in the rat. We feel assured of this not only because the presence

of such a layer was seen by Retterer (1892) but because we have seen it in sections from a guinea pig which we killed at this period, in which we also saw unmistakable evidence of the occurrence of the superficial epithelial layer which is the source of the epithelial cells of Stage One in the rat and probably also in the guinea pig. Stockard and Papanicolaou (1919, pp. 234–235) evidently saw this cornified layer, but interpreted it as the whole "epithelium separated from the underlying tissue." In several instances we also have found this layer shed *en masse* and preserving the form of the lumen of the vagina, and under the microscope satisfied ourselves that it consisted chiefly of a continuous layer of cornified cells, to which, however, there adhered large numbers of the epithelial cells characteristic of the next stage of the guinea pig, Stage 3 (old Stage Two). Whereas in the rat Stage Two is dry, in the guinea pig it is characterized by an abundance of mucous derived from the uterus.

Stage Three (old Stage Two of the guinea pig).—This stage is marked in both animals by the desquamation of large numbers of cellular elements to form a "cheesy mass" which in the rat consists of non-nucleated cornified cells derived from the cornified layer and probably the continued cornification of the next deeper, flattened stratified layers. In the guinea pig this "cheesy" material is made up of nucleated epithelial cells. In this animal the vaginal epithelium is very thick (more so than in the rat) and may well be considered as giving rise to the cheesy mass, after losing its stratum corneum *en masse,* by the rapid exfoliation without cornification of the deeper layers of cells until it later reaches the condition shown in their figure 17 (pl. 5, 1917). In both animals leucocytes collect under the epithelia of both uterus and vagina. It is in this stage, also, in both animals that ovulation occurs, perhaps a little earlier in the rat than in the guinea pig.

Stage Four (old Stage Three of guinea pig).—This stage is characterized in both forms by a sudden wave of leucocytic invasion of the mucosa in both vagina and uterus. In both there is continued destruction of these epithelia. In the rat the vaginal smear now consists of cornified cells and leucocytes, in the guinea pig of the corresponding epithelial cells and leucocytes. Destruction of the uterine epithelium in the rat goes on by a sort of vacuolar degeneration resulting in the loss of single cells rather than in the loss of large areas as in the guinea pig. This degeneration may, however, begin earlier in the rat.

Stage Five (old Stage Four of the guinea pig).—This stage is not represented in the rat by the appearance of blood externally, as in the guinea pig. In both cases regeneration begins, but whereas in the rat the vaginal epithelium is renewed from the deep layer which is never lost, and the uterine by mitosis of those cells remaining, in the guinea pig the vaginal mucosa is said to be regenerated from the portions in the deep folds, and the uterine from the uterine glands.

The interval is much longer in the guinea pig, both animals are quiescent during it, and in both the vaginal smear consists of epithelial cells and leucocytes.

It will be seen, then, that in spite of several divergencies in detail, there are the same fundamental changes in all the reproductive organs of both rat and guinea pig and the same correlations between these organs.

VI. THE ATTAINMENT OF SEXUAL MATURITY

A. Age at First Copulation

Sexual maturity must be defined as the earliest stage at which the animal is capable of producing young. Satisfactory evidence as to the advent of sexual maturity might be supposed to come from a knowledge of the date at which first litters are born in an animal colony in which the sexes are reared together. Under these circumstances it is unlikely that the first oestrus will be experienced by a female who does not have an opportunity to copulate. We present data upon this point in the subjoined curve A, page 53, which shows the earliest copulation known in the case of one hundred ninety-nine individuals. In some of these cases the date of copulation was calculated from the date at which the first litter was born, in other cases we had the opportunity, in our round of daily examinations, to observe the vaginal plug. It will be noted that most instances of the first known sexual congress occur in rats between sixty-three and one hundred and seven days of age; taking the entire data, the average age at the first coitus is 92.7 days.

It is apparent, however, that these data are neither necessary nor adequate to answer the question as to when ovulation begins in the rat, for it is conceivable that there could be some lack of correlation in the development of the ovaries and vagina or that typical oestrous desire is not sufficiently manifested at the inception of the first cycle to lead to mating.

B. Age at Establishment of Vaginal Orifice and at First Oestrous Changes in Vaginal Smear

We have consequently been led to examine with some care the time of establishment of the vaginal orifice and to correlate it so far as possible with the condition found in the ovaries. In immature animals the lumen of the vagina does not extend to the exterior, but is closed by what appears to be a thick membrane which comes to resemble a cicatrix by its glistening character and appears to become gradually thinner and thus ruptures. This rupture would appear to occur by the widening of a minute aperture centrally located in the vaginal membrane, but it often takes place in two minute apertures bilaterally placed, leaving a thin median cord of epithelial tissue extending across the vaginal orifice dorsoventrally. We have seen this cord persist until the first parturition.

In a large proportion of all normal animals (in our estimation in a little less than one-half) the actual breakdown of the vaginal membrane is correlated with the first oestrus and is heralded by a swelling of the lips of the incipient opening, just as is the case with the onset of Stage One in the oestrous cycles of the adult. In all these cases at the time of occurrence of the actual opening a sampling spatula withdraws a vaginal smear showing either Stage One or Two, i.e., it will indicate that the process of cornification has occurred and either the superficial, characteristic, small, nucleated cells of Stage One or the subjacent cornified cells of the following stage will be encountered. Unpublished observations by K. O. Haldeman show, in fact, that a cornification of the vaginal epithelium appears first in the solid epithelial core which extends from the vaginal lumen to the surface of the body. In this at separated intervals intra-epithelial vesicles are established. In cases where vaginal opening is coincident with cornified changes throughout the vagina and the animal is sacrificed twenty-four hours later, sections of the ovaries and oviducts will show a single set of corpora lutea, the first ones, and the first eggs in the Fallopian tube; and from what has been said of the long persistence of the corpora of ovulation we may be certain that no shortly preceding ovulation has occurred. Furthermore, animals killed before the opening of the vagina possess ovaries without any corpora whatever, but exhibiting merely follicles in various stages of growth and especially in stages of atresia. Ovulation therefore does not happen before the establishment of the vaginal orifice.

These circumstances indicate that the first ovulation and the opening of the vagina often stand in very close relation to each other, but it would be a mistake to take the latter as a criterion for the former. In fact, in animals in which the ovaries have been ablated at about the thirtieth day of life the vaginal orifice is established at about the usual time, so that this event need not stand in any relation to the first oestrus. In a considerable proportion of normal cases (as high as 40 per cent of all cases) the establishment of the vaginal orifice takes place before the first oestrus and hence by the breakdown of the closing membrane without cornification in the vagina; and for a few days the samples of the vaginal content will disclose a smear consisting only of scattered epithelial cells with a few leucocytes. Sections of the ovaries in these cases before the incidence of oestrus show that an ovulation has not yet occurred; but when the growth, cornification, and dehiscence of epithelium takes place and is finally disclosed by the vaginal smear, follicular ripening and ovulation are invariably found.

These first oestrous changes in the vaginal smear which are thus actually associated with the first ovulation, are not usually long delayed after the establishment of the vaginal orifice, though we have encountered isolated instances of great delay. As will be seen from table 12, the majority of those animals

which do not experience their first ovulation simultaneously with the establishment of the vaginal orifice, nevertheless do experience this ovulation within five days thereafter.

TABLE 12.

Showing chronological relation of first ovulation to opening of vagina in 193 rats.

	Instances	
Opening and first ovulation simultaneous	88	46%
Ovulation within the first 5 days after opening	43	22%
Ovulation 6 to 10 days after opening	22	11.5%
Ovulation 11 to 15 days after opening	20	
Ovulation 16 to 20 days after opening	4	
Ovulation 21 to 25 days after opening	7	
Ovulation 26 to 30 days after opening	5	
Ovulation over 30 days after opening	4	
Total	193	

Thus, while our studies would lead us to caution any one who would attempt to use the first establishment of the vaginal introitus as an index of maturity, they nevertheless demonstrate that the first oestrus occurs within a few days succeeding this event, and that the first oestrus is not peculiar in the respect that any lack of correlation exists between ovary and vagina, for the correlation is identical with that which we have shown to obtain throughout the sexual life of the adult, i.e., oestrous indications in the vaginal smear are from the beginning associated with the incidence of ovulation.

The second curve (B) shows the age at the time of establishment of the vaginal orifice in 466 individuals. It will be seen that the orifice is established in most cases between the fifty-third and one hundred and second day of life, the average age computed from our data being 76.5 days. In a group of carefully selected, well-kept stock the average age was even slightly higher—about eighty days. Other groups have given an average much lower, so that we must admit that our data must be considered merely as depicting the conditions obtaining in our colony during the time occupied by this study.

There are thus about ten or twelve days between the average age at first oestrus and the average age at first coitus, a fact which could perhaps find its readiest explanation in the coincidence of the latter date with the approximate time of occurrence of the second oestrus, for, as shown in table 13, the first oestrous cycle averages approximately ten days in length. In the majority of cases where the formation of the vaginal opening and the first oestrus are simultaneous the closing membrane is not ruptured until Stage Three is reached, by which time oestrus proper has passed. This may explain failure to breed at the first oestrus in this group.

A. ———— Curve showing distribution at five day intervals of ages at which first copulation occurred in 199 rats.

B. ———— Similar curve for ages at which the vaginal membrane ruptured in 466 rats.

A surprising fluctuation exists in the age of maturity. While the extremes observed (table 13) indicate that rats may mature as early as at 45, or as late as at 147 days, there is also a considerable variation in the average age of maturity of a unit lot of rats handled at one time in exactly the same way as regards food, space, etc., and the same applies to litter mates. Some indication of the way in which the nutritive factor might tend to influence our results is suggested in the careful records of the time of maturity of 70 rats in which the usual table scrap diet was supplemented by whole milk and in some instances

TABLE 13.

Age in days at opening of vagina and at first ovulation, and length in days of first four œstrous cycles in a group of 200 rats.

	Range	Average
Age at opening	34th-109th day	72d day of life
Age at first ovulation	45th-147th day	77th day of life
Length of first cycle	3-37 days	10 days
Length of second cycle	3-25 days	9 days
Length of third cycle	4-26 days	8.5 days
Length of fourth cycle	3-23 days	7.3 days

by still other articles of diet, notably one-half gram uncooked liver daily. In these animals the growth rate was notably accelerated, but the age at maturity averaged 84 days, so that it appears that a superior nutrition need not hasten the establishment of ovarian function.

VII. THE PHENOMENA OF REPRODUCTION AND THEIR EFFECT ON THE NORMAL RHYTHM

A. Pregnancy

1. LENGTH OF GESTATION

The exact length of gestation in the rat, which we would define as the time intervening between copulation and the occurrence of parturition, lies between 21.5 days and 22 days. In two hundred and one cases it was possible for us to know the period of gestation by quite accurate observations of the beginning and terminal event, that is, it was possible to know within eight hours when copulation took place by observations of the presence of the vaginal plug; and by placing animals in the obstetrical cage, which has been described earlier, the exact time of parturition was determined. In seventy-six of these cases (38 per cent) the time of gestation was within a few hours of 21.5 days; in one hundred and four cases (52 per cent) it was slightly less than 22 days. Table 14 shows the distribution of these instances of various gestation lengths. Some of these variations could be accounted for by variation in the relations between ovulation and copulation, as shown for embryos (p. 33). The longer ones must be attributed to other causes still unknown. In none of the above was the mother suckling a litter, a condition first shown by Daniel (1911) for mice, and King (1913) for rats, to influence greatly the span of gestation.

Table 14.

Length of period of gestation in the rat.
(Probable error 8 hours)

Length in days	Instances
20	1
21	9
21½	76 (38%)
22	104 (52%)
22½	2
23	7
24	2

Average of all gestations
21.8 days

2. PROPORTION OF OVA PRODUCING YOUNG

It is well known that resorption not infrequently occurs, even in fairly late stages of pregnancy. It is also conceivable that in many instances fertilization does not result or that some of the ova never become implanted. Data for the first time can now be presented giving an accurate idea of the per cent of eggs which do not produce young. From records of some 625 litters (table 15) it is found that the average number in the litter is 6.9, and that the mode also is 7. As previously stated (table 4), the average number of eggs found in one oviduct is 4.8, an average of 9.6 for the individual at each ovulation. If all were fertilized and could develop, the average expected size of a litter should be 9.6. According to these figures, somewhat less than one-third of the eggs liberated from the ovary never develop. To be more certain, the average size of the litters was determined for the same group of animals in which those fifty individuals were taken in which the eggs could be counted in the oviduct. The average size in this group of one hundred and fifty-six litters (table 16) is 6.4, making a mortality of exactly one-third of eggs matured and liberated from the ovary. It would be interesting and important to determine the effect of nutrition on the number of young produced and whether the number of eggs matured could be increased, and also possibly whether the amount of atresia in the ovary lessened. It is hoped to furnish such data at a later time. Such a problem should also be attacked from the standpoint of heredity.

TABLE 15.

Number of young per litter in the rat.

Number in litter	Instances
1	1
2	8
3	27
4	58
5	70
6	110
7	*114*
8	105
9	68
10	43
11	14
12	12
13	3
14	1

625 litters—average 6.9

TABLE 16.

Number of young per litter in same group of animals which furnished material for counts of eggs and corpora per ovulation.

Number in litter	Instances
1	2
2	5
3	9
4	13
5	20
6	*30*
7	28
8	21
9	18
10	7
11	2
12	1
156 litters—average 6.4	

3. EFFECT OF PREGNANCY ON THE REPRODUCTIVE ORGANS

The incidence of pregnancy has a peculiar interest to the student of the physiology of reproduction. Special changes occur in all the organs of reproduction, changes which owe their origin to the complex series of events characterized by coition, fertilization, implantation, and other characteristics of the pregnant state. The cause of these may in some measure be analyzed as we continue to know more of the specific rôle played by each component of the reproductive system.

Vaginal changes.

It has always been supposed that ovulation, as well as oestrus, is withheld in the case of all mammals during the period of gestation, but we are not aware of the existence of any rigid proof of this supposition. The demonstration that ovulation is always correlated with a precise set of changes in the histology of the mucous membranes of the genital tract, changes which can be detected in the living animal by means of the vaginal smear, gives us a chance to discover whether this "sign" of ovulation is ever encountered during the interval occupied by gestation. In our experience no oestrous changes occur in the cell content of the vaginal smear throughout the period of pregnancy. The smear is always typified by the occurrence of scanty epithelial cells and leucocytes, to which, as will be explained later, may be added red blood cells during the first three days of the third week. After the extensive exfoliation of cornified cells,

which begins with and immediately follows the copulatory act, no more cornified cells are encountered in the vaginal smear throughout gestation; nor do sections of the vagina taken at various times during pregnancy ever show a stratum corneum and the particular kind of typical, high, stratified, squamous epithelium which always goes with the possession of a cornified layer.

TABLE 17.

To show the time of first appearance of film of red blood corpuscles on the floor of the vagina near the cervix.

Days after fruitful copulation	12	13	14	15	Total
Number of instances in all of which the day of copulation was known	1	44	51	3	99
Number of instances in which the day of copulation was calculated from the day of littering	1	28	46	13	88
Total number of instances	2	72	97	16	187
Per cent	1%	39%	52%	8%	100%

On the other hand, the epithelium of the vagina during pregnancy comes to acquire a characteristic histology. This is also ultimately the case with the appearance to the naked eye of the vaginal mucosa, which usually about the fourteenth, and always by the sixteenth, day of gestation has a thick, velvety appearance quite unlike that seen in the non-pregnant condition. It is, indeed, at this time, also, that the vaginal speculum will disclose a bright red, bloody discoloration of the floor of the vagina, especially in its upper part near the cervix, a discoloration actually due to the presence of free blood upon the surface of the mucosa, as the careful removal of it by means of a swab, as well as the examination of it when removed by the spatula will show; so also sections (fig. 65, pl. IV) of the exudate *in situ*. It is extremely probable that we have to do here with a leakage of blood of uterine, and, presumably, placental origin, which has escaped through the cervical canal. In our opinion, this is the earliest infallible sign of pregnancy in the rat which may be detected in the living animal. It is highly interesting that once it appears it does not persist throughout the remainder of pregnancy, but is typically present for but three days (i.e., from the fourteenth to the seventeenth day), during which time it is hardly added to or renewed to any significant degree because the color changes from the bright red of fresh blood to a brown color through the well understood "ageing" of the blood pigment (table 17). This sign may rarely begin at the thirteenth day,

or, again, be present for the first time on the fifteenth day. It will remain for future work to detect just what changes in the pregnant uterus bring about this transitory hemorrhage.

We should turn now to what we have described as a characteristic histology for the vaginal mucosa of pregnancy. We may state that this mucous membrane shows in the middle layer of its epithelial cells a characteristic vacuolarization so extensive as to characterize the entire vaginal canal in the last week of pregnancy when the epithelium has also reached a considerable height. The change has not yet appeared on the eighth day (fig. 63, pl. IV), but is evident by the tenth (fig. 64, pl. IV), and reaches its greatest expression from the sixteenth (fig. 66, pl. IV) to the twentieth (fig. 67, pl. IV) day of gestation. It is detectable first in the epithelium of the mucosal folds near the cervix and vaginal portion of the cervical canal. It is very remarkable that a squamous transformation of the superficial cell layers does not ensue, but that the surface cells instead of being flattened are always fairly voluminous structures, columnar in form. Many mitoses are present and these account readily for the growth in the height of the epithelium, especially when we bear in mind that but slight loss occurs from the inconsiderable dehiscence. Along with the absence of cyclical changes, heat and copulation do not occur during pregnancy. However, our records show that two animals copulated during gestation, one on the fourth and fourteenth days, and the other on the sixteenth day.

Ovarian changes.

(1) *Suspension of ovulation.* Our demonstration that oestrous changes in the vaginal mucosa do not occur during pregnancy might, in view of the invariable correspondence which we have observed between the ovary and genital tract, be taken to indicate that ovulation is also withheld, but the final conclusive proof of the suspension of ovulation is afforded by the employment of a selective vital dye for the corpora lutea. Some time ago one of us reported the elective behavior of certain vital benzidine dyes with respect to ovarian tissue, and called attention in particular to the fact that deposits of these dyes (administered to the living animal) were especially accumulated in the macrophages of atretic follicles and to a lesser extent in the lutein cells of most corpora lutea.

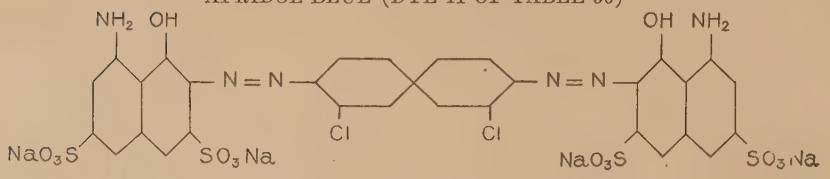
We have experimented with eighty-three dyestuffs (most of them benzidine compounds) which by reason of their constitution might be expected to serve as vital dyes. Their behavior toward the ovary has been summarized in table 36 of the Appendix. We may remark here that most of these substances are competent to act as vital stains in the sense in which that term is usually employed for the effects produced by Trypan Blue and other benzidine dyes. In the case of those dyes which produced a deep vital tinging of the animal as a whole a widespread vital staining was obtained—an effect on the cells of the

connective tissue throughout the body and on certain of the endothelia—whereas in the case of those which do not tinge the body as a whole there is nevertheless always at the site of their injection an identical effect on the same cells, although one which is restricted to the immediate locality. Most of these eighty-three substances, which are thus ingested and stored by the macrophages of the living animal in granular form, are to be understood as segregated and concentrated in vacuoles which Evans and Scott have called "the segregation apparatus" of the cells, most of which may be specially formed for this purpose. The cells of the mesothelium, though differing from the endothelia, the fibroblasts, and the tissue macrophages, also participate in a characteristic way in this reaction.

It is highly interesting to note that the most successful general vital stains, in the sense in which this word has usually been employed, fail to give the most satisfactory effects on the true lutein cells of the corpus luteum. Many of the dyes, such as Trypan Blue, or the first two dyes listed in table 36, are invariably brilliant general vital stains, and, while they tinge deeply the corpora lutea as a whole, microscopic investigation of the fresh tissue shows at once that the color is due largely to deposits in the mesothelial ovarian covering (the germinative epithelium), and that the true lutein cells are conspicuous in contrast thereto by the possession of only very pale deposits of the dye. Indeed, Dye 26 of the table gives an intense crimson stain to the entire animal, but produces granular deposits in the corpora lutea too inconspicuous or pale for detection! Nevertheless, it is undeniable that some general deep vital stains produce more easily distinguishable, deeper colored deposits in the lutein cells; and we would instance here dyes 9, 10, 14, 15, 41, and 59 of the table.

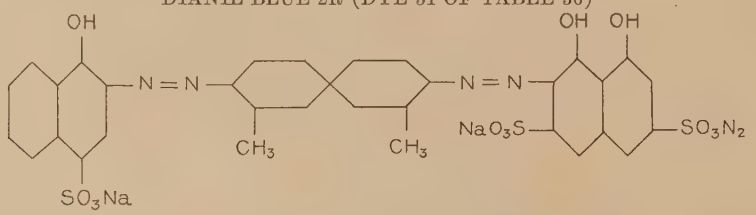
We have discovered that two dyestuffs whose intensity of reaction with most vitally stainable cells is inferior to the above deep general dyes, nevertheless give the most distinctive and best colored deposits in the true lutein elements, and, above all, give the only deposits which, having these qualities, are also capable of perfect histological fixation. It is apparent, therefore, that the reaction toward these dyes on the part of the corpus luteum cells is somewhat different in character from that displayed by the great mass of those cells in the connective tissues and endothelia which are stained vitally, especially the so-called "macrophage cells." These two vital stains, which, while giving merely a weak tinging of the animal as a whole, nevertheless so clearly mark with deposits the corpus luteum cells, are *Afridol Blue,* a combination of dichlorbenzidine diazotized and coupled in alkaline solution with two molecules of 1.8 amidonaphthol 3.6 disulfonic acid,

AFRIDOL BLUE (DYE 11 OF TABLE 36)



and *Dianil Blue 2R,* the combination of ortho-tolidine diazotized and coupled in alkaline solution with one molecule of 1.8 dioxynaphthaline 3.6 disulfonic acid (chromotrope acid) and one molecule of alpha-naphthol 4 monosulfonic acid (Neville-Winther acid).

DIANIL BLUE 2R (DYE 51 OF TABLE 36)



Afridol Blue produced by far the deepest colored deposits in the lutein cells, but after prolonged experimentation with it we decided that even in small doses its general toxic effect was so evident as to jeopardize one's confidence that one is dealing with normal phenomena after its employment. In particular the reaction of this substance on the organs of the reproductive system justified a further search for a less toxic dye, for, administered during pregnancy, Afridol Blue was often responsible for abortion or resorption of the conceptus, and in other cases appeared equally responsible for an extensive follicular atresia so frequently encountered in the ovary after its administration.

The second dye, Dianil Blue 2R, was remarkably adapted to our purpose. This dye, in aqueous solution a deep reddish-blue, or bluish-violet, can be administered at any time in the sexual history of an animal in doses so large as to fill the peritoneal cavity and on four or five successive days without serious toxic effect and usually, indeed, without any detectable toxic effect on the animal.[3]

It is but fair to state, however, that several points in the technique of the intraperitoneal administration of the dye are worthy of note if one would be sure to disturb least the normal physiology of the experimental animal. The animal should not be submitted to the toxicity of even transient anaesthesia and the dye administration should follow rather than precede the daily feeding time. It is somewhat surprising, but true, that animals which have been handled daily are sufficiently tolerant of manipulation to be grasped tightly in one hand by the skin of the back while the hypodermic needle punctures the ventral abdominal wall and the injection is completed. The distress occasioned by a voluminous intraperitoneal injection (e.g., 5 c.c.) is so great that rats will not partake of their daily ration if the injection is made within a short time of the feeding hour, but if the injection follows the feeding hour by a one to three hour interval the full stomach does not empty itself *per os,* and a normal nutrition is thus assured.

[3] Reference has already been made to this vital ovarian dye in other work issued from this laboratory by Corner and Hurni and by Monroe Sutter, to whom we have introduced its employment.

If, on the day following three or four daily intraperitoneal injections of this dye, an animal be sacrificed and the corpora lutea which happen to be present in the ovary examined in the fresh by cutting out a minute piece of the corpus with iridectomy scissors and crushing this in salt solution under a cover glass, it will be seen that each cell of the germinal epithelium contains considerable numbers of deep blue vital dye "granules" and that all the true lutein cells also contain smaller numbers of more scattered, minute, spherical vital dye "granules." The "dye granules" are of almost exactly the same dimensions as the lipoid spherules which occur in the cell at this time and with which they are

Fig. 50. Outline drawing of fresh ovary of Rat 3514 (before fixing in Benda's fluid) to show the corpora of pregnancy (deep blue), of lactation (light blue), and of ovulation (2 sets, colorless). Sections in figure 51, plate VIII, and figure 89, plate IX, are taken along lines AB and CD. Given dye during lactation; young weaned on the 25th day; the next Stage One occurred 3 days later; the following day in Stage Two a glass rod was introduced into the cervix; fifteen days later the animal was killed in Stage Three of the first recurring oestrus; eggs of this ovulation were found in the distal folds of the oviduct.

admixed. The dye deposits, or granules, are fixed readily in formol, Bouin's fluid, or Zenker's fluid, but, unfortunately, are not visible in material preserved in mixtures containing osmic acid. In view of this fact, in all cases in which we desired to examine the lipoid character of corpora lutea which were at the same time marked vitally by means of these dye deposits, the ovary was sketched in salt solution under the camera lucida and the position of all corpora noted, the vitally stained corpora being especially labeled so that their identification in section was easy (figs. 50; 51, pl. VIII, and 89, pl. IX). In the ovaries of animals treated with such a vital dye only certain of the corpora are affected in the way in which we have just described; other corpora, which we have reason to believe

are younger, are unaffected. A careful test of this question in animals whose sexual and oestrous history was accurately known did, in fact, show us that corpora lutea, whether of ovulation, gestation, or lactation, do not readily receive deposits of the vital dye during the first three or four days of their life (figs. 59 and 60, pl. VI). It is apparent, therefore, that the vital-stainability of the corpus cell, by which we mean its permeability to the vital dye substance and its segregation and storage of the same, is characteristic only of corpus cells of a certain age (fig. 60, pl. VI), the younger corpus cells (fig. 59, pl. VI) maintaining a completely refractory behavior toward penetration by the dye, just as was the case when they constituted the granulosa cells of the ripe follicle. Older lutein cells, then, constitute a different physico-chemical protoplasmic system and the vital dye gives a clear demonstration of how this change may occur in the cytomorphosis of a single cell.

Corpora lutea which are produced subsequent to dye administration are always unstained. They never have traces of the vital dye, so that absorption of the coloring matter from an old corpus on the part of a fresh structure does not take place; nor have we evidence that vitally stained macrophages, which, as we know, are often present in the corpora and have considerable powers of migration, ever wander from a stained to an unstained corpus. The vital staining method is hence of inestimable value in the case of an animal like the rat, where the most diverse conditions pertain with regard to the age and number of corpora lutea present at any one time.

Since late in gestation the corpora can be deeply stained with the vital dye they can thus be distinguished with certainty from the corpora produced at subsequent ovulations which do not possess the dye, but which might resemble them in other ways. As will be shown farther on, this method is also essential for ready identification of the peculiar corpora lutea found in nursing animals, the *corpora lutea of lactation,* and has a more extended value in the fact that in cases where ovulation has been suspended after dye administration irrefutable proof of this is seen in the fact that no unstained corpora arise in the ovary during the experimental interval. By this means we were able to demonstrate that the prolonged pause in oestrus experienced by rats after coitus or after the introduction of a rod into the cervical canal is accompanied by suspension of ovulation.

If four or five cubic centimeters of a 1 per cent aqueous solution of this dyestuff be administered intraperitoneally on the four successive days represented by the fourteenth, fifteenth, sixteenth, and seventeenth days of gestation and the animal bred again at the incidence of the first oestrus (i.e., that which occurs on the day of parturition) and killed at the time of second parturition, one may obtain conclusive evidence of the absence of all ovulations during the second pregnancy. The ovaries of animals treated in this way contain only two

kinds, or sets, of corpora lutea, one, the corpora of the first pregnancy, the only ones now present which are vitally stained and the only ones which were hence present at the time of dye dosage, and the other set, the corpora of the second pregnancy, normal in color, i.e., uninfluenced by the vital stain, and consequently to be interpreted as those which were formed subsequent to dye treatment. These corpora formed subsequent to dye treatment correspond in number with those which could be expected from a single ovulation (the average number of corpora lutea graviditatis found in one hundred cases of pregnancy being 4.9), and, moreover, in size and all histological characteristics, including the character of their lipoid deposit, they are identical and hence of the same age or ovulation, and constitute the corpora resulting from the ovulation on the day of parturition transformed into the corpora of gestation on account of the second conception at this oestrus.

The above facts appear to us to estalbish beyond peradventure the fact that ovulation is entirely suspended during pregnancy, and, furthermore, demonstrate that the corpora of pregnancy result from the continued growth and enlargement of the particular corpora of ovulation from the follicles of which the ova were fertilized.

(2) *Size and characteristics of the corpora lutea graviditatis.* Until almost the middle of pregnancy, i.e., during the first nine or ten days after a fruitful copulation, the corpora lutea do not differ in outspoken characteristics from the corpora of ovulation in their size or in the number, size, and distribution of the lipoid granules (fig. 68, pl. VII, 14th day); distinctive characteristics of the corpora of pregnancy are hence lacking. But after the tenth day of gestation continued slow growth of these corpora permits them to attain dimensions never found in the corpora of ovulation, dimensions which may approach two millimeters in diameter and which toward the end of gestation (sixteenth to twentieth day) are almost always from 1.75 to 1.9 millimeters. This enlargement is brought about to some extent, of course, by growth of the extra-luteal constituents of the corpus, but it is produced chiefly, if not entirely, by growth of the individual lutein cells comprising the structure. This final enlargement is not shared by corpora of the preceding ovulation; in other words, the latter are not capable of responding in this way, the corpora of gestation being produced from the single set of follicles the eggs of which were fertilized. If copulation and pregnancy had not occurred the above set of follicles would have given rise to corpora of ovulation.

This is easily proved in a case in which an animal, which was given dye during gestation, was allowed, after parturition, to pass one cycle, and was then bred at the second oestrus. The corpora of the first gestation, though small, were blue and could clearly be distinguished from the large corpora of the last gestation while all the others were of the one ovulation between.

TABLE 18.

Comparison of different kinds of corpora lutea with regard to size of corpora and size and amount of lipoid granules.

Corpus luteum of	Length of functional period in days	Characteristics during first cycle					Characteristics during end of first and beginning of second cycle	Characteristics during third cycle
Ovulation	4-5	Rat No.	3533				3533	454
		Days	2				4	11 pp.
		Diameter	1 mm.				1.05-1.2	1.1
		Lipoid:						
		Size*	1-2				1-7 (2x9)	1-7
		Amount	moderate				moderately heavy	Very moderate
		Fig.	48				49	
Copulation or pseudo pregnancy	8-16 (about)	Rat No.		3639	3587		3514	
		Days		10	13		15 (eggs in oviduct)	
		Diameter		1.35	1.1		1.4	
		Lipoid:						
		Size*		1-3	1-5 (8)		1-9	
		Amount		moderate	moderate		very heavy	
		Fig.		(placentoma)			88	
Pregnancy	21½-22	Rat No.	3479	3558 3471	447		428	452
		Days	4	10 16	21		Within 12 hrs. pp.	7½ pp.
		Diameter	1.15	1.22 1.8	1.7		1.75	1.23
		Lipoid:						
		Size*	1-2 or 3	1-3	1-9		1-9 (2x9)	1-9
		Amount	moderate	moderate	moderate		Very heavy	Moderate
		Fig.					69, 70	71
Lactation	21-45	Rat No.	366	373	425	3629	360	358
		Days	9	14	21	2 after weaning	4 after weaning (eggs in oviduct)	8 after weaning
		Diameter	1.03	1.36	1.45	1.2	1.2	1.05
		Lipoid:						
		Size*	½	½	½	1-5 (8 & 9)	2-9	1-7, 9
		Amount	Very moderate	moderate	moderate	Very heavy	Very heavy	Moderately heavy

*The size is indicated by numbers which are given to the ocular spots shown in figure 46. See also p. 38. The extremes include the deeper half of the corpora. Figures in parenthesis are of relatively few, large, lipoid granules.

Marked changes in the lipoid morphology of the lutein cells of the corpora of pregnancy, changes which we believe are indicative of cessation or diminution of function, take place at the end of pregnancy; and within twelve hours post-partum (figs. 69 and 70, pl. VII), the enlargement of lipoid globules and hence great inequality in the size of these bodies as well as their increase in amount is very evident (see table 18). In the case of animals which are not permitted to suckle their young and which are not bred on the day of parturition (i.e., cases in which pregnancy is succeeded by normal ovulation), these cells of the corpus of pregnancy continue to become filled with lipoid deposits until the time of occurrence of the next ovulation following that on the day of

parturition, when, just as we have shown to be the case with the corpora of ovulation, the lipoid in the rapidly ageing lutein cells undergoes a second phase, a phase of marked diminution (fig. 71, pl. VII).

(3) *Persistence of the corpora lutea graviditatis.* The method of staining vitally the corpora of pregnancy enabled us, of course, to follow the continued regression and final time of disappearance of these structures in animals killed at various time intervals after the gestation in which the corpora were stained. Although functional impairment of these bodies may already be detected at the close of gestation, they persist in the ovary as do the corpora of ovulation, but for a longer time. If the animal be sacrificed and the ovary examined four days after parturition, i.e., after the occurrence of the first post-partum ovulation (fig. 56, pl. VI), the corpora of pregnancy are seen to be stained vitally even more deeply than is the case in animals which are examined immediately after the conclusion of actual dye administration or at parturition (fig. 55, pl. VI). This apparent increase in vital stain of the corpora is, in our estimation, due to the fact that with the formation of subsequent corpora lutea, which protrude somewhat from the ovary, those of gestation slowly diminish and in this process their covering tissue, the germinative epithelium, to a certain extent follows them. Since the cells of the germinative epithelium are stained more deeply than the subjacent lutein cells, they make an even more emphatic color impression when now concentrated in the smaller area. This method of identifying cells by means of the vital stain thus shows the retention of the identity of the mesothelial tissue overlying a particular corpus and demonstrates that most of the epithelial covering of the new corpora represents a new formation rather than a shift in position and stretching out of the mesothelial tissue which is vitally stained and once covered the old corpora.

Twenty days after parturition the corpora lutea of gestation are still substantial structures—somewhat over a millimeter in diameter—though the number of their proper lutein elements is considerably diminished. Even about the fiftieth day after parturition (fig. 58, pl. VI) the corpora of gestation are about half a millimeter in diameter and contain numbers of lutein cells in which always a few small lipoid granules are visible, though at this time by other methods the structure would hardly be recognizable as that of a preëxisting corpus, for the inconspicuous lutein cells are separated by connective tissue in which vitally stained macrophages are prominent. By means of the vital dye method, however, it is possible to detect small areas in the ovary, usually seen from the surface as deep blue spots, which are the last vestiges of the corpora of gestation about one hundred and twenty-five days after the day of parturition. Though most of this small area is considerably beneath the surface of the ovary, still part of it usually reaches the surface; the macroscopic color, of course, is due solely to the vitally stained germinal epithelium and to macroph-

ages which have completely engulfed all lutein elements with their vital dye contents. The corpora of pregnancy have thus been identified in the series of cases represented by figures 72, 83, plate VIII; 53, 89, plate IX; 54, 73, 82, plate X.

TABLE 19.

Length of first post-partum œstrous cycle when suckling was prevented.

Days	Instances	
3	2	
4	2	
5	11	27
6	4	
7	7	
8	1	
9	0	
10	1	
11	0	
12	0	
13	3	
14	2	

27 cases average 5.5
33 cases average 6.9 days

TABLE 20.

Length of first post-partum œstrous cycle when young were nursed less than twelve hours.

Days	Instances	
4	1	
5	4	
6	3	*20
7	5	
8	7	
15	1	

*20 cases average 6.6 days.
21 cases average 7 days.

(4) *Effect of the corpora lutea gravidatis on other corpora lutea.* We have already stated that on the twentieth day of the gestation period only a single conspicuous set of corpora lutea are found, the corpora of gestation, but that on the twentieth day post-partum, when normal ovulations have occurred, not less than thirty corpora are present in the ovary. It is apparent, therefore, that there is not only a suspension of ovulation during pregnancy but also profound retrogression and resorption of all preëxisting corpora lutea. All corpora of ovulation, then, regress, even those formed at the oestrous period immediately preceding that at which mating was permitted.

The more substantial structures represented by corpora of a preceding gestation do not diminish to the same extent as do those of ovulation.

In animals kept isolated, normal ovulation should succeed parturition at regular four or five day intervals, but we have found that the first oestrous cycle following parturition, as determined by the vaginal smear, tends to be somewhat longer. Table 19, compiled from 33 cases, shows that this cycle is 6.9 days in length.

TABLE 21.

Effect of number of young suckled on length of interval between weaning and first subsequent ovulation. Young weaned at 21 days.

No. in litter	No. of litters	Average increase in weight of litter*	Average length of interval
2	5	51 grams	3.4 days
4	7	59.4 grams	4 days
6	10	91 grams	6.9 days
7	8	100 grams	9 days
8	7	87 grams	7.7 days
9	7	88 grams	6.7 days
10	4	111 grams	10 days
11	2	106 grams	11 days
12	2	83.5 grams	10.5 days
13	2	94.5 grams	14.5 days

*The litters were weighed at birth and at 14 days, and not at 21 days because during the last week the young are likely to partake of other food. We are indebted to Miss G. J. White for this data.

B. LACTATION

1. EFFECT OF LACTATION UPON THE VAGINA

Young rats may usually be weaned after twenty-one days of suckling, but if left with the mother the duration of lactation may be double this time. Daily microscopic examination of the vaginal smear from suckling mothers shows the persistence of the condition characterizing the dioestrous interval, i.e., leucocytes and scattered epithelial cells. In the light of the invariable correspondence which we have found to obtain between ovulation and the character of the vaginal smear it might be inferred that the above evidence indicates that ovulation is suspended during suckling. This evidence is strengthened by the recurrence of the usual typical oestrous changes in the vaginal smear, which take place in about seven days after the suckling young are removed. The recurrence of the oestrous changes in the vaginal smear may take place from three to twelve days after the removal of the young and would appear in general to have some dependence on the number of young suckled. Oestrus recurs somewhat more tardily when the litter is large and the drain occasioned by lactation is consequently greater, an indication of which may be obtained from

the weight increase of the litter as a whole (table 21). When the young remain with the mother, oestrous changes in the vaginal smear will occur spontaneously at some time between the twenty-fifth and fortieth day after parturition.

The histology of the vaginal mucosa during lactation shows that not only are the cornified changes associated with oestrus absent, but that a very considerable and characteristic reduction in the height of the epithelium occurs. While in the second day of lactation (fig. 74, pl. IV) this mucous membrane may still consist of four or five simple cell layers, by the fourth (fig. 75, pl. V) day more than three layers are seldom encountered, and on the sixteenth day (fig. 76, pl. V), when lactation may be assumed to be at its height, most of the vaginal mucosa is actually reduced in its epithelial investiture to two cell layers. *The superficial cells of the vaginal mucosa in lactation are cubical or low cylindrical elements.* The strict dependence of this characteristic epithelium upon the performance of the mammary glands could be illustrated in no more striking way than by the transformation of it which may take place within forty-eight hours (fig. 77, pl. V) after the removal of the young when a high, stratified, squamous epithelium results.

2. EFFECT OF LACTATION UPON THE OVARY

Suspension of ovulation.

It is well understood that as a rule ovulation is suspended during lactation, an idea entirely verified by the findings in the vaginal smear which we have just detailed. However, proof that ovulation is inhibited during lactation may be furnished by staining the corpora of gestation vitally at some time during the last week of pregnancy and examining the animal so treated at any time during the first three weeks of lactation, when only a single unstained set of corpora lutea are found—the corpora of lactation. Furthermore, if in animals treated in this way we wait until the vaginal changes indicate the advent of the first oestrus after nursing, we find in these ovaries, besides the stained corpora of gestation and the unstained corpora of lactation, only the freshly formed corpora of this ovulation. It is, therefore, established that lactation inhibits ovulation.

Corpora lutea lactationis.

The corpora which we have designated the corpora lutea of lactation deserve distinction from the corpora of gestation and of ovulation on account of the characteristic picture of their lipoid bodies, for these uniformly distributed spherules or granules (fig. 79, pl. VII), which brown or blacken with osmic acid, are smaller than those possessed by the corpora of ovulation or of gestation. The corpora of lactation result from the post-partum ovulation occurring within twenty-

four hours of parturition, as is also proved by marking the only other corpora which may be confused with them, the corpora of gestation, with the vital dye. Thus the lactating act, which has been in force at least twelve or fourteen days, imposes on the corpora of the post-partum ovulation a characteristic morphology of the lipoid deposits which, though slightly larger than the minute fat-bodies sometimes found in the granulosa cells before follicular rupture, are never permitted to attain the average size which is already reached by the lipoids of the ordinary corpora of ovulation (fig. 48, pl. VII) or gestation (fig. 68, pl. VII) within twenty-four to thirty hours after their formation. The corpora lutea of lactation, however, can not be easily distinguished in other ways from those of ovulation or gestation; they continue to grow beyond the size attained by the corpora of ovulation, although they do not attain the dimensions of the corpora lutea graviditatis; they apparently reach their maximum size at some time between the fourteenth and twentieth day of suckling, when a diameter of 1.4 millimeters may be attained.

We have already mentioned the fact that, to judge by changes in the lipoids, the impairment of the corpora lutea of ovulation barely precedes the next oestrus, but so short is the time interval involved between the beginning of these changes in the ordinary corpora (ovulation) and the next oestrus that one might have difficulty in assuring himself as to which of the two events is anterior. We have also shown that this difficulty is not experienced in the case of the regression of the corpora of gestation, for well marked changes in the lipoids of these cells are always elegantly displayed in the first twelve hours after the birth of a litter and before the post-partum ovulation has occurred.

The relation of lutein cell degeneration to the incidence of the next oestrus is even clearer in the case of the corpora of lactation. Cessation of lactation provokes lipoid changes within the lactation corpora within twenty-four hours so that we have in our hands the means of bringing this about simply by removal of the young. One day after the litter has been removed the lactation lutein cells no longer possess their fine, dustlike lipoid granules (fig. 79, pl. VII), but have lipoid granules which are already larger than those found in the healthy corpora of ovulation or those of pregnancy. (See also table 18.) Two days after litters have been removed the lactation lutein cells contain still larger and markedly irregular lipoid masses, about like those at four days (fig. 80, pl. VII), and yet the next oestrus does not usually occur until at least four days reckoned from the removal of the young, by which time the lutein cells are gorged with lipoid granules crowded together and beginning to fuse into large masses, especially in most of the cells in the inner half of the corpus.

One fact is evident from the sequence of phenomena which we have just described, namely, that degeneration of functional corpora lutea does not itself bring on the next oestrus, even though there can be no doubt, of course, that the

occurrence of oestrus is effectually restrained when corpora are functioning actively and that this restraining influence is released on the advent of decay of the corpus. It is evident that the fundamental cause of oestrus, whatever it may be, can not again operate so quickly after a period of lactation, and especially the lactation of a large litter, as it can under the normal conditions characterized merely by the usual succession of ovulations. It would be important to assemble quantitative information here on the influence of lactation on the repression of the growth of follicles or on the production of follicular atresia. It is somewhat surprising that the termination of pregnancy, where, moreover, we have to do with a great bulk of active lutein tissue, permits a speedier recurrence of oestrus and ovulation, as witnessed by the prompt post-partum ovulation, and we can only explain this by the assumption that lutein cell decay has occurred anterior to parturition.

Effect of lactation on preceding corpora lutea graviditatis.

We have already called attention to the fact that on the last days of gestation the corpora lutea of pregnancy are the only corpora present in the ovary, the corpora of all preceding ovulations having been brought to atrophy and resorption. A somewhat similar effect is seen in lactation, during which the corpora lutea of the preceding pregnancy tend to disappear more rapidly than under conditions in which the young are removed as soon as born and pregnancy is succeeded merely by normal ovulation.

When animals are placed in the obstetrical cage during the last day of pregnancy so that lactation is prevented, the young being separated from the mother automatically as soon as born, and when after these conditions the ovary is examined from the twentieth to twenty-third day post-partum, normal ovulations having intervened, the corpora of pregnancy are still seen as fairly substantial structures barely over a millimeter in diameter with many, though atrophied, lutein cells in which are moderate lipoid deposits (Rat 325).

When the corpora of pregnancy are examined at a similar time interval, which has, however, been occupied by vigorous lactation, they are somewhat smaller in size and contain either no lipoid or very fine lipoid deposits in their lutein cells, which are still scantier in number and, indeed, in some cases have disappeared (Rat 425).

C. Copulation

1. Normal copulation and the formation of the vaginal plug

From the preceding sections it will be seen that the length of the ovarian cycle may be influenced by various conditions. It is interesting that the act of copulation itself also exerts a similar though not so great an influence. An attempt to analyze this phenomenon has led to the discovery of a still more remarkable set of facts which can best be presented after a discussion of normal copulation.

The manner of copulation in rodents and especially in the rat has been described by earlier investigators: Lataste, Steinach, Kirkham. Yet it may not be

amiss to present a more complete account of certain aspects of the subject. The condition of the female is of importance because, as has been pointed out earlier in the paper, copulation can take place only when the female is in heat, which, as we have shown, occurs typically in the transition from Stage One to Stage Two of the vaginal smear and in the early part of Stage Two. It will be recalled that at this time the lips of the external orifice of the vagina are slightly swollen and tend to make the opening more prominent; the vaginal mucosa is dryish, and covered with the thick cornified layer which, as Lataste long ago pointed out, probably serves as a protection. The cornification is accompanied by and possibly is the cause of a somewhat disagreeable odor which not unlikely is a means whereby the male is made aware of the condition of the female, for, as is common among animals, recognition may be chiefly carried on through the olfactory sense. The condition of the vaginal smear is sufficient to enable the investigator to determine in nearly all cases when the female will copulate and is of the greatest service in breeding animals for purposes of embryological study, for it is only necessary to look through a colony to pick out those individuals which will usually copulate with little delay. In investigating the relation between the stages of the oestrous cycle and heat it was, of course, necessary to resort to the use of males, for many individuals show no signs that enable one to recognize the heat condition. Careful observation and considerable experience has enabled us, however, to detect oestrous quickly in most cases by the behavior of the female when placed with males. It might be mentioned parenthetically that the female rat, unlike other mammals, only rarely attempts to play the part of the male in riding other females. When such a female in heat is placed in a large cage in which males are kept she does not remain quietly in a corner, but is more or less active, constantly moving about and often keeping near the males. If she is not in heat she does not respond in any way to the male and after a short time is ignored. On the other hand, if in heat, her reactions are characteristic and consist in running about more or less intermittently with a curious hopping gait, stopping when a male succeeds in making an attempt, when the back is flattened in a characteristic way; indeed, the back is bent so that it becomes concave with the tail up and the head back. Under these circumstances and also frequently when she is hopping about, the head is shaken so that the ears quiver with a fine vibrating movement. It is an interesting fact that the position of slight opisthotonos taken by the female at coition may often be elicited by inserting rather firmly a speculum into the vagina while the animal is held in the hand, but the elicitation of this reaction has not resulted on account of contact of the speculum with the actual cervical canal, and unlike cervical stimulation it does not delay the appearance of the next oestrous cycle. This can not be considered a sign of heat, for in some individuals the animal will respond in this way at any time during the cycle. Nevertheless, it is usually characteristic only of the period of oestrus.

One of the most striking features of the copulatory act in rats is the great celerity with which attempts are made and actual insemination is accomplished by males, the movements of the posterior part of the body being very rapid. After each attempt, of which there may be very many before a fruitful coitus is consummated, the male almost invariably rolls back on to his hind quarters and licks his genitals. To an inexperienced person these attempts may be easily mistaken for a true copulation. But experience and vigilant observation will show that the latter differs from the former in being slightly more prolonged and in the male more often rising on his hind feet instead of promptly rolling backwards. He is usually quiet for a time afterwards, but as such inactivity often follows abortive coitus it is not a reliable sign. The only certain indication is the presence of a "plug" in the vagina of the female immediately after. Unless this is looked for at once it may be lost if the female is left with males for the reason that males may copulate as many as five times within a short period of time, and with the same female, one or two attempts being sufficient to dislodge a plug from the vagina. If the female is isolated the plug will usually remain in the vagina about twelve hours, occasionally as long as twenty-four hours. Of course, in any case, the actual finding of sperm in the vagina is the crucial test of insemination. But in our experience a plug is never unaccompanied by sperm.

The vaginal plug of rodents has been the subject of study by a number of investigators with regard to its structure, origin, use, and chemical composition. Although its occurrence was known before the time of Lataste and has been mentioned by many others since, he seems to have been the first to give it careful attention in his papers from 1882 to 1893. Lataste called the plug the *bouchon vaginale,* and was aware that most of it came from the seminal vesicle. He believed, however, that the outer part, or "envelope," was a contribution from the vagina of the substance which we now know to be the cornified layer which in some instances was so abundant as to give rise to a large mass, in consistency not unlike a plug. Lataste's conception of an outer "envelope" or coat formed by the vagina is easily explained by the fact that most plugs which are allowed to remain in position until they fall out do carry with them various portions of the cornified layer of the vagina, to which the plug is adhering tightly, the cornified layers being in the act of dehiscence at this time. Indeed, since one may occasionally even withdraw a more or less perfect cast of the vagina in the form of a sheet of cornified cells which constitutes the entire stratum corneum removed *en masse,* it is easy to believe that many of the vaginal plugs which Lataste examined must have contained an external coating with these cornified cells. Walker (1910) has shown conclusively that the plug is derived from the secretion of the seminal vesicle coagulated by the secretion of a coagulating gland (as the writers can confirm). As sperm are being expelled the secretions

of the seminal vesicles and of the coagulating gland mix and evidently "set" the ejaculated mass, making it adhere tightly to any object with which it is in contact.

One will have no difficulty in seeing the formation of a plug that may be withdrawn from the penis of an animal killed by a blow on the head. On the tip of such a plug is a small mass of spermatozoa, of which use has been made in artificial insemination. It might also be noted that occasionally a plug is found attached to the hair of the female. All these plugs have the same general shape. It is apparent that at the moment of ejaculation the plug is moulded to fit the vaginal lumen, especially at the cervix, for the plug is constant in shape

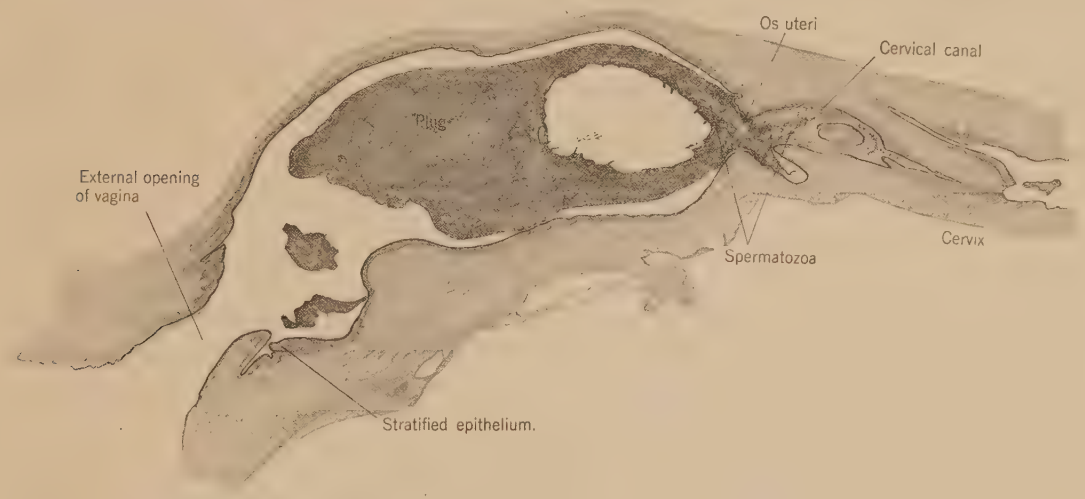


Fig. 84. Diagram of longitudinal section of vagina with plug *in situ* extending a short distance into the cervical canal. Rat 3580. × 6.

and has two little prolongations, each of which extends into one of the cervical canals (fig. 84). The plug adheres so closely to the cornified layer of the vaginal mucosa (fig. 85, pl. XI) that it is not easily distinguishable in section. The plug is naturally loosened so that it may be dislodged from the vagina when this cornified layer becomes stripped off spontaneously in every oestrous cycle, as described in an earlier section. In fact, the cornified layer may not only be considered a protection but also a means of insuring loss of the plug after it has performed its function. Sometimes two or even three plugs remain in the vagina at the same time.

The plug is not a solid body, but contains cavities, especially at its deeper end, these cavities being filled with spermatozoa. One of the obvious functions of the plug apparently is to confine spermatozoa between itself and the uterus as though to give abundant opportunity for them to make their way into the uterus.

Immediately or very soon after copulation the uterus is found to contain a considerable amount of dark reddish fluid in which there are enormous numbers of spermatozoa. The presence of this fluid has been noted by other investigators (Sobotta for the mouse and Königstein for the rat), but its relation to the fluid produced during Stage One has never before been understood. It will be recalled that in the first stage of the oestrous changes the uterus becomes distended with fluid. But this fluid, unlike that after copulation, is always, in normal animals, clear and colorless. That the bloody nature after copulation is not due to the spermatozoa is proved by the fact that after copulation by vasectomized males, which are unable to eject spermatozoa, the same bloody appearance is to be noted. It is probably caused by the act of copulation itself.

The male is capable of copulating and forming these plugs at any time of the day or night, but is most active at night. This last peculiarity may be the reason for a certain amount of apparent infertility in the case of females which are in heat for the first time in the morning hours and are past the heat period by night, when sexual activity in the male is at its height.

In the breeding of rats for embryos for any purpose except highly accurate studies on age, the observation of the plug is of the greatest value in saving time. It is only necessary to examine daily with the aid of a speculum the vaginae of mated females early in the morning or late at night for plugs from which to date the approximate age of embryos. Many animals can in this way be examined quickly. From the variations found in the development of embryos, even of the same copulation age, a fact which has been pointed out in earlier parts of this paper, it will be evident that some variation is unavoidable. But the inexactness in estimating the development of embryos dated from the finding of a plug is but slightly greater than if copulations be actually observed. Some plugs will prove to be infertile, of course, just as is the case with observed copulations. The following figures are of interest in this connection. One hundred and ninety-eight healthy young females were confined in several lots in each case with an almost equal number of healthy young males, their litter mates. During a fixed time interval we made daily observations with the vaginal speculum at an early hour each morning. Two hundred and forty-four gestations resulted during this time interval and three weeks thereafter, but during the same time interval only one hundred and fifty-two plugs had remained in the vagina long enough to be observed in the morning round of examinations, and of these one hundred and fifty-two plugs, one hundred and thirty-four, or slightly over 88 per cent, were followed by conception. It is evident that by this method alone, regardless of the fact that many plugs fall out during the night, a copulation record may be obtained for many pregnancies, and in almost 90 per cent of all cases where the plug has remained *in situ* until morning, gestation will follow. On the other hand, it is to be pointed out that in a colony handled in this way we will have almost as many pregnancies which are not preceded by the observation of a vaginal plug *in situ* the following morning. In our particular set of animals, of the two hundred and forty-four gestations resulting, in only one hundred and thirty-four cases was the plug seen the next morning, i.e., in only 54.8 per cent of the total gestations was it possible to obtain a record of the copulation by this method of observation. This figure might be higher if twice daily examinations were made.[4]

[4] This is a far more expeditious method of dating embryos than by actually observing copulation, and might well be an important factor in deciding whether to use rats or pigs for laboratory work in embryology.

2. INFERTILE COPULATIONS

Diseased females with normal males.

It has just been pointed out that a small percentage of copulations (or plugs) are sterile. It had been found early in this investigation that some of such females when left with males would copulate only at fairly long intervals without ever becoming pregnant. Such females turned out to be sterile because of an infection of the oviduct which occluded the lumen, but did not prevent ovulation in most cases. The reason for these long cycles is undoubtedly the same as for those which will be next reported.

TABLE 22.

Infertile females mated with normal males with intervals between plugs.

Designation of Animals	Intervals in days between plugs in order of occurrence
158	7, 3, 3, 3, 3, 5, 6
159	13, 26, 14, 13, 13, 12, 12, 13
160	17, 13, 16, 15
161	14, 13, 13
197	12
198	10, 13
201	20, 13, 4
202	27
205	17, 23, 19, 16
206	15, 25, 13, 27, 12, 13, 23
208	13, 25, 13, 12, 14
209	4, 2, 17, 16, 18, 16, 18, 17, 40, 19
211	14, 10, 7
212	3, 3, 3, 4, 5
214	17, 16, 14, 15, 15, 15, 14, 14, 12, 4, 14
247	18, 17, 17
249	28, 13
294	17, 19
310	13
316	13, 13
339	5, 12, 4, 6, 7, 2, 2, 3, 3
342	7, 8, 4, 3
361	9, 15, 11, 4, 4, 3, 2, 4, 3, 4
386	6, 10, 8, 3, 5
393	9, 3, 7
394	23, 5, 10, 12, 13
395	9
396	14, 13, 14
404	18, 16
406	14, 12
407	12, 13
431	10
441	14
450	12, 12, 12
1188	12, 26, 13, 10

Normal females with vasectomized males.

In the course of a study of living eggs by one of us (Long, 1912), it was found that when females were allowed to mate with males rendered sterile by vasectomy, eggs could always be procured in the oviducts during the day on the morning of which a plug was found. Such males, from which a small piece of each vas deferens was resected, could copulate in a perfectly normal manner, but were impotent to inseminate a female. It seemed then as though the use of these males furnished an excellent method of studying the occurrence of ovulation since if copulation took place only at the time of ovulation the presence of the plug, uncomplicated by pregnancy, would be taken as an indication of ovulation. Females were accordingly placed each day with a new vasectomized male to give the maximum opportunity for coitus and careful daily observations made. The results of many of these cases are shown in table 23, from

TABLE 23.

Nine normal females mated with vasectomized males, with intervals between plugs.

Series Number	Intervals between plugs (in days) in chronological order
142	12, 24, 11
301	13, 13
305	19, 14, 14, 16
309	7, 4, 13, 13
410	12, 28
413	12, 16, 27, 15, 26, 16
422	14, 4, 15, 16, 16
423	8, 4, 4, 4, 12, 16, 16
440	10, 4, 4, 18, 15, 14, 14

which we would have to deduce an ovulation period that could not be harmonized with the earlier supposed cycle of ten days, nor with the true later discovered cycle of four to five days. This long copulation interval of healthy females with vasectomized males was in complete accord with the results on the copulation of sterile females possessing healthy ovaries but occluded oviducts. Furthermore, in both kinds of cases the vaginal smear showed a delay in the appearance of the next oestrus (table 24).

3. MECHANICAL STIMULATION OF CERVICAL CANAL

It was then that the suggestion offered itself that perhaps the secretions of the male accessory glands might be of importance in this connection. Previous studies had been made by other authors on the influence of prostate secretion on the activity of the spermatozoa and on fertility (Hirokawa, Iwanoff, Steinach, Walker), none on the influence on the female. Since it seemed possible that the plug itself, perhaps by being partly dissolved and absorbed, might exert an influence independent of the act of copulation, an attempt was made to mix

TABLE 24.

Length of œstrous cycles following infertile copulations, or copulations with vasectomized males, as determined by vaginal smears.

Length of cycle in days	Number of cases	
3	1	
5	2	
6	1	
9	3	
10	5	
11	14	
12	8	
13	5	51 cases
14	4	average
15	5	13.1
16	3	days
17	2	
18	2	
19	3	
58 cases—average 12.4 days		

the two secretions responsible for its formation in the vagina. Although a sort of plug was formed, but not successfully, no lengthening of the cycle followed. The only other possibility that suggested itself was the secretion of the prostate (table 25). Accordingly, the secretion obtained by triturating the bull's prostate gland, and also extracts of it in Ringer's solution, were injected into the uterus through the cervical canal by means of a glass syringe, using the method employed by one of us in earlier work (Long and Mark, 1911) with the result that the following cycle was longer than normal by several days, a very gratifying sequel. Controls consisting of injection of the same substance into the peritoneal cavity were negative. The same result followed, however, when Ringer's solution was injected into the uterus and in a few cases when the syringe was inserted but obstructed in some way so that the contents could not be expelled into the uterus. In fact, it was then discovered that merely inserting into the uterus a small glass rod had the same result! (table 26). In all of these cases the uterus was reached, of course, by traversing the cervical canal. As further experiments proved, the same results follow when the rod is inserted only about one millimeter into the cervical canal (table 26). This was done by using a slender brass rod with a small collar near the tip to prevent the rod being pushed in too far. It was apparent, therefore, that not only was it unnecessary to traverse the entire cervical canal but that the characteristic effect of cervical simulation could be elicited from that part of the canal known to possess the same stratified squamous epithelium as the vagina. The prolongation of the cycle follows only when the instrument is introduced during Stages One to Three

(table 26), that is, during, and shortly before and after, the period of heat. This undoubtedly explains the action of the plug in prolonging the cycle since the plug fits tightly into the vagina and extends a short distance into the cervix.

This discovery of the profound influence on the length of the oestrous cycle of stimulating the tip of the cervix of the uterus by the mere momentary introduction into it of a slender rod is one of the most remarkable reactions known to us. The questions which naturally arise are what is the explanation of the mechanism of this striking phenomenon, and what is its significance? A partial answer to these questions can be given when dealing with the subjects of deciduoma formation and ovarian transplantation.

TABLE 25.

Effects of administering various substances *in utero* or intraperitoneally.

Figures in italics indicate the length of the period immediately following treatment, the other figures the length of periods preceding and following this.

Designation of animal		
	Secretion of rat prostate. *In utero.*	
2412	4, 4, *15*, 3, 4	
	Extract of rat prostate in Ringer's solution. *In utero.* About .2cc per dose.	
2010	5, 4, *17*, 5, 8	
2332	4, 4, *15*, 4, 6	
2395	6, 5, *13*, 4, 4	
2518	3, *20*, 5, 3	
	Extract of rat prostate in Ringer's solution. Intraperitoneally.	
2383	5, 6, *5*, 6, 6	Given in middle of period. 1cc.
2395	4, 4, *6*, 5, 11	Given on second day of period. .2cc.
2412	4, 4, *4*, 3, 2	Given on second day of period. .6cc.
2557	5, 5, 6	Given on second day of period. 1.0cc.
2547	5, 5, *19*, 8, 3	.2cc.
2568	*4*, 5, 4	.2cc.
2897	*6*, 4, 5	.2cc.
3096	5, 6, *7*, 7, 7	.2cc.
	.9% salt solution intraperitoneally. Doses of 4cc.	
2208	5, 5, *8*, 3, 6	
2383	5, 4, *5*, 6, 6	
2502	5, 5, *5*	
2537	5, 5, *10*, 11, 8	
2571	9, 4, *4*, 4, 5	

Extract of seminal vesicle of bull in Ringer's solution. In utero. ca .2cc

2319	8, 7, *21*, 12, 5	Extract heated 1½ minutes at 60-62° C.
2518	3, 5, *5*, 4, 6	Given on second day of period (about 24 hours after Stage 2).
2586	4, 5, *11*, 5, 6	
2850	6, 5, *13*, 5, 5	Extract heated 1½ minutes at 60-62° C.
2861	5, *10*, 5, 4	
2896	8, 6, *6*, 6, 7	Extract heated 1½ minutes at 60-62° C.
2908	4, 4, *7*, 5, 8	Extract heated 1½ minutes at 60-62° C. Given late in Stage 2.
2930	5, *12*, 4, 5	
2975	5, 5, *11*, 4, 4	
3017	5, 18, *12*, 4, 4	Given on second day of period (about 24 hours after Stage 2).

Extract of prostate of bull in Ringer's solution. *In utero.*

2384	5, 5, *12*, 4, 4
2502	5, 6, *10*, 4, 5
2568	4, 5, *16*, 5, 4
2917	3, 6, *12*, 4, 4
3011	6, 4, *11*, 3, 5
3095	6, 4, *10*, 4, 6

Ringer's solution in utero.

2319	12, 5, *21*	
2332	5, 5, *17*	
2350	5, 6, *12*, 3, 9	Failed to inject solution, but introduced syringe.
2351	8, 4, *12*, 4, 4	Failed to inject solution, but introduced syringe.
2384	4, 3, *13*, 4, 4	
2486	5, 5, *16*, 3, 5, 4, 3, *17*, 4	
2518	4, 5, *17*	
2542	5, 6, *11*, 4, 3, 6, 6, *12*, 4, 6	
2547	5, 5, *6*	Tried to inject solution but failed.
2560	6, 5, *11*, 6	
2568	4, 4, *19*, 5	
2586	9, 5, *14*, 4	
2739	4, 4, *13*	
2850	5, 4, *14*, 4, 4	
2861	8, 4, *18*, 4	
2896	4, 4, *14*, 5	
2908	5, 4, *14*, 5	
2912	5, *6*	
2930	4, 8, *21*	
2942	5, *12*, 5, 4, 6, 6, *11*, 4, 4	
2956	5, 4, *12*, 6	
2960	4, 5, *11*, 5, 4	
2963	6, 5, *15*, 6	
2968	5, 5, *14*, 4	
2975	*21*	
2981	8, 7, *15*	
3011	4, 4, *21*	
3014	4, 4, *3*, 14, 4, 4, 5, *15*, 5	
3017	4, 5, *12*, 4, 4	
3095	4, 4, *12*, 7, 5	

TABLE 26.

Length of œstrous cycle in days after introducing a glass rod once into the cervix a distance of about 10 mm. and allowing it to remain for a few seconds, during Stages **1**, **2** or **3**. Figures in italics indicate the length of the period immediately following treatment; the other figures the length of periods preceding and following this.

Designation of animal	Periods	Remarks
2350	5, *15*, 4	
2384	4, *16*, 4	
2486	4, *16*, 4	
2518	4, *16*, 5	
2542	*11*, 5	
2850	4, *18*, 4	
2861	4, *15*, 4	
2896	5, *12*, 5	
2908	5, *12*, 5	
2942	4, *13*, 4	
2956	6, *12*, 4	
2960	4, *19*, 4	
2963	6, *11*, 4	
2968	4, *20*, 3	
3011	4, *16*, 5	
3014	5, *16*, 4	
3017	4, *16*, 3	
3028	7, *18*, 5	
3073	6, *19*, 9	
3079	8, *15*, 5	
3086	5, *13*, 6	
3579	4, *14*, 4	
	4, *5*, 4	Rod inserted only 1 mm.
	4, *4*	Rod inserted only 1 mm.
	16, 4	Rod inserted only 2 mm.
3599	4, *19*, 4	
	4, *21*, 5	Rod inserted only 1 mm.
3641	*11*	
3642	*11*	
3723	4, *11*, 5	Rod inserted only 3 mm.
3724	4, *13*, 4	
	6, *5*, *11*, 7	Rod inserted only 1 mm.
3726	4, *12*, 5	Rod inserted only 3 mm.
3729	*17*	
3730	*13*	
3746	7, *13*, 4	
	4, *10*	
	4, *12*, 4	
	4, *11*, *13*, 4	
	4, *10*, 4	
	4, *11*, 4	
3747	7, *10*, 9	Rod inserted only 3 mm.
	9, *10*, 6	
	6, *10*, 4	
	6, *12*, 5	
	5, *14*, 4	
	4, *12*, *11*, 4	
	4, *13*, 4	
3753	13, *12*, 5	
	4, *5*, 4	
	4, *4*, *4*, 5	
	5, *4*, *4*, 3	
	4, *4*, 4	
	4, *4*, *16*, 5	
	4, *4*, 4	
3754	11, *9*, 5	
	5, *5*, 7	
	7, *5*, 5	
	4, *6*, 4	
	4, *14*, 8	
3755	8, *4*, *5*, 6	
	5, *14*	Failed to introduce rod
	4, *4*, 4	
3756	7, *5*, 4	Failed to introduce rod
	4, *5*, 4	Failed to introduce rod
	4, *13*, 3	
3820	16, *4*, 4	
	4, *13*	Rod inserted only 1 mm.
	20, 12	
3883	5, *13*, 19, 11, 10	
	6, *17*, 6	
	6, *15*, 4	
	4, *4*, *14*, 5	
	4, *4*, 8	
3886	5, *4*, *4*	
	4, *4*, 4	
3887	7, *13*, *13*, 4	
	4, *6*, 7	
3888	5, *6*, 4	
	6, *20*, 5	
	3, *4*	
3890	6, *4*, *12*, 4	
	4, *4*, 4	
3893	4, *4*, *9*, 16	
	7, *18*, 5	
	5, *15*, 5	
	5, *17*, 5	
3895	6, *10*, 10	
3896	6, *3*	
	3, *4*, *12*, *16*, 5	
	4, *18*, 4	
	4, *20*, 9	
	5, *18*, 5	
3898	4, *17*, 4	

TABLE 26—(*Concluded*)

Designation of animal	Periods	Remarks
3748	4, *8*, 3	Rod inserted only 3 mm.
	3, *12*, 5	
	4, *15*	
	4, *13*, 5	
3749	9, *4*, 10	
	4, *11*, 4	
3750	5, *8*, *6*, 4	
	9, *3*	
	4, *16*, 4	
	4, *5*, 8	
	8, *3*, *13*, 3	
	4, *13*, 4	
	5, *4*, 4	
3751	3, *15*, 4	Failed to introduce rod
	4, *11*, 4	Failed to introduce rod
	4, *14*, 4	
	4, *5*	
	4, *15*, 5	
	5, *12*, 5	
3898	4, *23*, *16*, 4	
	4, *22*, 4	
	4, *19*, 5	
3899	5, *14*, *4*, *4*, 4	
	5, *5*, *10*, 4	
	4, *19*, 5	
	5, *6*, *4*, 6	
3900	4, *3*, 10	
	3, *20*, 4	
	4, *13*, 4	
	4, *14*, 4	
	4, *13*, 4	
	3, *16*, 4	
3901	4, *4*, 5	
	4, *4*, *14*, 3	
	4, *4*, *6*, *23*, 4	
	4, *4*, *20*, 4	
4869	8, *8*, 6	
	4, *4*, *25*, 4	
4870	4, *15*, 4	
	4, *15*, 4	Rod inserted only 1 mm.
	4, *14*, 5	Rod inserted only 1 mm.
4872	4, *15*, 4	
	4, *16*, 4	Rod inserted only 1 mm.
	4, *13*, 4	Rod inserted only 1 mm.
4873	5, *20*, 6	Rod inserted only 1 mm.
	6, *17*	Rod inserted only 1 mm.
4874	4, *20*, 5	
	4, *4*, 18	Rod inserted only 1 mm.
4875	4, *13*, 5	
	4, *17*, 4	Rod inserted only 1 mm.
	4, *8*, 5	Rod inserted only 1 mm.
2856	5, *14*, 4	Rod introduced during interval
2739	4, *4*, 15	Rod introduced during interval
2896	5, *4*, 5	Rod introduced during interval
2908	5, *12*, 4	Rod ntroduced during interval
2942	4, *4*	Rod introduced during interval
2956	5, *6*, 4	Rod introduced during Stage 3
2963	4, *14*	Rod introduced during interval
2975	4, *5*, 21	Rod introduced during interval
3014	4, *12*, 4	Rod introduced during interval
3046	6, *15*, 5	Rod introduced during interval
3054	4, *17*	Rod introduced during interval
3064	5, *11*	Rod introduced during interval
3072	6, *4*, 13	Rod introduced during interval
3082	7, *6*, 14	Rod introduced during interval

Rats 3746 to 3756 had mammary glands excised (see table 31).
Rats 3579, 3599, 3820, and 4869 had uteri removed (see table 25).
Rats 3883 to 3901 were used for ovarian transplantations (see table 27).

4. PROOF THAT COPULATION AND CERVICAL STIMULATION INDUCE A CONDITION OF "PSEUDO-PREGNANCY"

At first sight the remarkable effect of copulation or of cervical stimulation in delaying the appearance of the next oestrus and ovulation would appear to be unexplainable, for these procedures would appear thus only to express themselves some days after their occurrence and such a belated physiological response, or effect, would be difficult to understand. It would be easier to understand the delay of an immediately impending ovulation produced by nervous or other influences or the acceleration of that ovulation by direct nervous or vasomotor means, but, as we have shown, copulation or cervical stimulation seems not to influence the progression of oestrous changes and ovulation at the time, but only the time of appearance of the next oestrous events. *The entire matter becomes more intelligible, however, when we discover that the effect of copulation and cervical stimulation upon the reproductive system is immediate, although its manifestation is deferred. The stimulus produces a condition which justifies the designation "pseudo-pregnancy."*

Effect upon the vagina.

During the ten or twelve day interval which elapses after infertile coitus or after stimulation of the cervical mucosa, the vaginal smear indicates that we have to do with the condition of prolonged dioestrum. During this time merely leucocytes and scattered epithelial cells are present in the vaginal smear. Furthermore, sections of the vagina confirm the inference which the smear would give regarding the absence of cornification changes in the mucosal epithelium. The vaginal mucosa does not increase in the characteristic way in height and stratification as it does immediately preceding oestrus, but, on the other hand, begins slowly to undergo changes which we have already described as characteristic for the condition of pregnancy, the main features of which are the occurrence of cuboidal or cylindrical surface cells and of vacuolization in the intermediate cell layers. Our material indicates that these changes are not apt to be so pronounced in animals killed at any time interval after copulation or cervical stimulation as they are at a corresponding time in pregnancy; nor have they, in our experience, progressed much beyond what is found on the tenth day of pregnancy at the most. The last statement may be amplified by the statement that the entire vaginal mucosa after cervical stimulation does not undergo the "pregnancy" changes, but that only the upper portions of it near the cervix do so. Figure 86, plate V, shows the condition of the vaginal mucosa in folds near the cervix on the thirteenth day after insertion of a small glass rod into the cervical canal. The histological picture is not quite so far advanced as that of the thirteenth day of pregnancy, but, on the other hand, would seem

to be further along than that which may be found on the tenth day of pregnancy (fig. 64, pl. IV) when, as we have already stated, these changes begin in this area of the vagina, thenceforth increasing and advancing downward in the viscus (Rat 3587).

It is apparent from the above that infertile copulation or cervical stimulation brings on conditions in the vagina identical with those occurring in the early days of pregnancy. The condition has consequently been called by us "pseudo-pregnancy," a term which has already been employed by Hill and O'Donoghue to characterize the sequelae of normal ovulation in the marsupials. It would be highly interesting to find out whether in these primtive mammals coitus with vasectomized males might prolong and possibly heighten the conspicuous changes in pseudo-pregnancy. We may, however, at once admit that we have not been able to obtain these effects in the guinea pig, where oestrus is not delayed by infertile copulation, and so would refer again to the conjecture previously made in this paper that this remarkable effect of coitus in the rat is either peculiar to this animal or to those which like it have an oestrous cycle so short that the tubal journey undertaken by the fertilized ova would not be completed and the uterus reached before another spontaneous oestrus would occur to prevent their implantation and thus destroy them.

Effect upon the ovary.

Studies on the ovaries of animals which have been submitted to cervical stimulation or coitus with vasectomized males show the persistence and continued slow growth of the corpora lutea produced at the oestrus when the experiment was inaugurated, i.e., the suspension of oestrus behavior on the part of the animal and of oestrous changes in the vaginal mucosa is correlated with the suspension of ovulation and the continued function of the last formed corpora lutea. These structures do not increase in size so rapidly as do the corpora lutea of gestation or lactation, but nevertheless they do attain diameters of from 1.2 to 1.4 millimeters. During most of the prolonged dioestrous interval following coitus or cervical stimulation the corpora retain a lipoid content identical with that of the healthy corpora of ovulation or pregnancy (fig. 87, pl. VII, and table 18, p. 64). At the end of such an interval the lipoid granules begin to increase somewhat in size and number and resemble the change accompanying the normal corpora of ovulation at the onset of the next oestrus (fig. 88, pl. VII). Here, again, consequently, we have a clear instance of the final degeneration of corpora lutea preceding the occurrence of oestrus.

The proof of the suspension of ovulation both after copulation by vasectomized males and after stimulation by mechanical means is easily carried out by the use of vital dye. Dye was administered in several cases during pregnancy; soon after parturition the animal was allowed to copulate with a vasec-

tomized male, and some fifteen days later, when she would copulate again, was killed and the ovaries preserved. In such ovaries are to be found only two sets of corpora, one stained blue (the corpora of pregnancy) and the other colorless and consisting of bodies all of the same character (the corpora of copulation). If ovulation had occurred during the long interval there should have been corpora constituting more than one set, distinguishable from each other by difference in lipoid content. Similar conditions are found and the same reasoning applies if dye is given during lactation, the cervix stimulated at the first oestrous stages following weaning and the animal killed at the next oestrus.

The structure of what might be called the corpus of copulation or of stimulation or pseudo-pregnancy does not differ in its essentials from that of ovulation and gestation. (See table 18, p. 64.) It will be seen from this brief account that the corpus of copulation or stimulation is in a sense one of ovulation partially transformed into one of pregnancy, the transformation not being complete because the forces or stimuli operating late in pregnancy are lacking here. Those stimuli to be found in the condition of the uterus as the result of the presence of developing embryos, so far can not be duplicated even by the formation of deciduomata as described further on. Consequently the cycle comes to an end before complete transformation is possible. From the nature both of the ovary and the vagina the condition resulting from stimulation of the cervix is justly called "pseudo-pregnancy."

D. Production of Deciduomata

Robert Frank was the first to ascertain that the experimental production of deciduomata, first discovered by Leo Loeb in the guinea pig, can be obtained in rats, and Corner and Warren in this laboratory have been able to confirm Frank's statements. Indeed, it appeared to the latter observers that deciduomata could be produced with peculiar ease in this species inasmuch as practically all their experiments were successful. They were led to assign their success to the constant presence in the ovary of an abundant amount of lutein tissue, using Loeb's hypothesis of the dependability of deciduoma production upon active corpora. Our experience is not in accord with this, although, as will be shown later on, it can probably be utilized as an explanation of such results. We may state at once that we have not been able to produce deciduomata in animals which are experiencing normal four day ovulation cycles. On the other hand, typical tumors of this sort, often of considerable size, may be produced under most conditions which involve a delay in ovulation. (Figs. 90, 91, pl. I; 92 to 95, pl. XI.) The present account has already demonstrated some physiological and some experimental conditions in which such delayed ovulation occurs, viz., after copulation or stimulation of the cervix, during lactation, and in the case of animals which have barely reached maturity.

The foreign body uniformly employed in our experiments for deciduomata production consisted of loops of black silk thread inserted in either horn of the uterus in such a way that they passed clearly into the lumen and out again to be tied loosely (fig. 90; compare with fig. 91, pl. I). Although the studies of other investigators have indicated that so simple a thing as trauma alone would be adequate to elicit a deciduomatous response, the thread has the further advantage of leaving one in no uncertainty as to the exact site of uterine injury, an advantage of some consequence in enabling one to be sure that a completely negative response has ensued when the specimen is examined microscopically.

As an answer to the objection that so grave a thing as the administration of an anaesthetic and the opening of the body cavity with handling of and perhaps a trauma of the abdominal viscera would itself delay ovulation and give us an abnormal cycle, we may state that our operations, which were carried out with all of the care and celerity possible, showed that, when so conducted, the procedures themselves seldom interrupt appreciably the onset of the next oestrus.

Irritation of the uterine mucosa by injury or by the presence of threads does not affect the length of the oestrous cycle. This is in striking contrast to the profound effect produced by the simple stimulation of the cervix. It would seem to mean that the portion of the uterus that will respond in this way is very narrowly localized to the part normally affected by the vaginal plug.

1. DURING NORMAL OVULATION CYCLES

In twelve cases at various times during the oestrous cycle we placed silk threads in either horn of the uterus, examining the organs again at the time of onset of the next oestrus. In no case was any deciduoma-like tumor produced (table 27). Further experience has inclined us to attribute two causes for this uniformly negative result. First, we now believe that the uterine epithelium is not sufficiently sensitive to produce a typical deciduomatous response until the cycle is at least three days old. Secondly, if the experiment be carried out this late in a normal ovulation cycle another oestrus will supervene within a day, effectually destroying the freshly forming tumors.

That fertilization of the ova at any particular ovulation time leads to the immediate suspension of succeeding oestrous cycles has, of course, been known for a long time, but it might be supposed that this rule would not hold in animals like the rat which have so short an oestrous cycle that it is less than the time required for the transit of the fertilized ovum through the Fallopian tube and the establishment of uterine implantation. Data of other observers (cf. Huber, et al.) indicate that the developing ova of the rat reach the uterine lumen about four days after copulation and remain a day or two before implantation. It is

thus conceivable that in the rat another oestrous cycle could occur before actual implantation of the developing embryo, were the occurrence of implantation the only cause for withholding the next oestrous cycle after fertilization, but we have shown that copulation withholds the next oestrus. Now this effect of copulation seen in the rat may itself be unique and may with good propriety be looked upon as necessary to the successful implantation of the fertilized and developing ovum, for, as we shall show later, there is good reason for believing that if oestrus did occur the uterine degenerative changes would effectually prevent implantation or bring to decay and resorption the freshly implanted eggs.

TABLE 27.

Operations for the production of placentomata during the normal cycle.

Designation of animal	Operated upon during	Killed days after operation	
3539	Stage 1-2	3	Killed in long Stage 3. No swelling; few decidual cells.
3596	Stage 2	4	Negative. Killed in next Stage 2. Uterus distended.
3530	Stage 4	8	Negative. Killed in next Stage 2.
3536	Stage 4 (end)	8	Negative. Killed in next Stage 4.
3581	Stage 4 (end)	4	Negative. Killed in next Stage 1-2. Uterus distended with fluid.
3523	12 hrs. after Stage 4	6	Negative. Killed in next Stage 4.
3528	First 24 hrs. of interval	8	Negative. Killed in next Stage 2.
3533	First 24 hrs. of interval	5	Negative. Killed in early next interval.
3584	First 48 hrs. after Stage 4	4	Negative. Killed in early Stage 4.
4026	3 days after Stage 2	7	Negative. Observed 2 days after operation. No cycle.
3617	2 days post-partum	4	Negative. Cycle not known. Left horn larger.
3631	5 days post-partum	4	Negative. Killed after new Stage 2.

In many animals with a longer cycle the next expected oestrus would occur after implantation were well established. We have found that in at least one of these cases, the guinea pig, copulation itself does not defer oestrus. These facts fit with the conception that in these animals foetal hormones are from the beginning responsible for the suspension of oestrus during pregnancy. It is our belief that while in the inseminated rat the initial inhibition of oestrus is due to cervical stimulation, the continued absence of cycles is here also the effect of foetal tissue.

TABLE 28.

Operations for the production of placentomata during pseudopregnancy.

Designation of animal	Time of stimulation	Operation days after Stage 2	Observed days after operation	Killed days after operation	Findings
3585	Stage 2	2		4	Slight enlargements.
3611	Stage 2	2	6. New loops	6 after last opr.	Negative. No cycles.
3614	Stage 2	2	6. New loops	6 after last opr.	Negative. No cycles.
3634	Stage 2	2	6. New loops	6 after last opr.	Negative. No cycle.
3732	Stage 2	2		6	Negative. Killed after new Stage 2.
4077	Stage 2	3		6	Negative. No cycle.
3630	Stage 2	4		2	Very slight swelling. Few decidual cells.
3636	Stage 2	4		2	Very slight enlargement. Few decidual cells.
3597	Stage 2	4		4	Moderate enlargements.
3639	Stage 2	4		6	Great enlargements. 7 mm.
3640	Stage 2	4		6	Great enlargements. 6 mm.
4447	Stage 2	4		6	Negative. No cycle.
4230	Stage 2	6		4	Negative. No cycle.
3613	Stage 2	6		4	Negative. Killed on day of new Stage 1-2.
3578	Stage 1	6		4	Negative. Killed on Stage 2.
3641	Stage 2	6		5	Negative. Killed on new Stage 2.
3642	Stage 2	6		5	Negative. Killed on new Stage 1-2.
4117	Stage 2	6		6	Negative. No cycle.
4076	Stage 2	6		6	Negative. No cycle.
3729	Stage 2		7. Large swelling	4 after observation	Negative. Killed on new Stage 2.
3730	Stage 2	6	7. Cornified	4 after observation	Negative. Killed after new Stage 2.
3731	Stage 2	6	7. Cornified	4 after observation	Negative.
54	Stage 2	6		7	Negative. No cycle.
4041	Stage 2	8		2	Negative. No cycle.
4257	Stage 2	8		21	Negative. Two sets of œstrous stages elapsed after operation. Cervix stimulated at last Stage One. Killed 6 days later.
3576	12 hrs. post part.	4		4	Negative.
3598	18 hrs. post part.	2		5	Negative.
3624	4 and 9 hrs. post part.	4½ hrs.		4	Negative.
3447	Stage 2	4 after stim.		6	Negative. Ovaries removed at oper.
3548	Stage 1-2	4 after stim.		6	Negative. Ovaries removed at oper.

2. DURING THE PAUSE FOLLOWING CERVICAL STIMULATION ("PSEUDO-PREGNANCY")

Inasmuch as our studies had taught us that after coitus or stimulation of the cervical canal the reproductive organs are in a condition resembling that of pregnancy, it was apparent that we had conditions which would enable us to test whether or not the response of the uterine epithelium to a foreign body was superior in character to that which we had been able to discover during normal cycles. Accordingly, at Stages One and Two a slender glass rod was introduced into the cervical canal of some thirty animals and at various times thereafter silk threads passed through the uterine walls and lumen and tied in position, the animals being sacrificed at various time intervals thereafter. Table 28 shows the result of these operations, which, in brief, is that if the operation is done on the fourth day after cervical stimulation and the animal sacrificed from the fourth to seventh day thereafter, providing no oestrus has recurred, excellent deciduomatous tumors well over a centimeter in diameter may be produced.

In no case after the incidence of the next oestrus is a tumor of this sort ever found, even though the time of operation and autopsy would otherwise lead one to expect that the response had taken place. We were therefore very definitely of the impression that the changes in the uterus associated with oestrus evidently brought these tumors to sudden atrophy and resorption, and we were confirmed in this interpretation by an experiment in which we were actually able to satisfy ourselves by laparotomy and inspection that typical deciduomata were actually present just before the onset of oestrus. The animal was closed up and killed at the incidence of the next oestrus. The uterus showed no evidence of the previously prominent uterine tumors and the sections no deciduomatous tissue (Rat 3729).

It is interesting that a thread after being placed in the uterus in one cycle never calls forth a response in the next cycle. A number of cases (not listed) prove this. The explanation would seem to lie in the fact that the threads become covered by a growth of cells. It has been found that in tissue cultures silk forms an excellent substratum along which cells may creep. Such a cell covering perhaps protects the surrounding tissue from the foreign body and hence from further irritation. Applying this idea to the failures following early operation, the suggestion presents itself that in early operation the threads became covered and so were prevented from exerting an influence at the appropriate time a little later.

3. DURING LACTATION

Under natural conditions the greatest delay in ovulation is, of course, that occasioned by the period of lactation which may not infrequently be prolonged to thirty days. It hence occurred to us that ideal conditions for the production and growth of deciduomata must exist during lactation. Here is a span of time greater than any other, even including the period of gestation, in which the ovary had been proved by us to refrain from discharge of ova and to nourish a set of large corpora lutea. We consequently placed silk loops in the uterus of twenty rats at various time intervals after the day of littering and while they were nursing normal sized, healthy litters, sacrificed them at various time intervals thereafter, as shown in table 29.

TABLE 29.

Operation for the production of placentomata during lactation.

Designation of animal	Operation days postpartum	Killed days after operation	Number of young suckled	Findings
3638	4	4	3-9	Some evidence of placentomata having degenerated. Suckling irregular.
4680	4	6	6	Large swellings. (5-6 mm.)
4671	4	6	6	Large swellings. (5 mm.)
4670	4	8	9	Large swellings. (5-6 mm.)
4681	4	10	6	Negative.
69	4	11	7	Negative.
59	5	11	5	Negative. No decidual cells in sections.
3637	6	4	8	Large swellings (4.25 mm.)
3591	7	4	8	Definite enlargements. (2.5 mm.)
3620	8	4	5+	Definite enlargements. (3 mm.)
3623	10	4		Large swellings. (4-5 mm.)
4678	10	8	4	Large swellings. (6 mm.)
4691	10	6	7	Negative, but left horn distended in region of threads.
4679	10	6	3	Negative; left horn slightly larger.
4672	11	6	5	Small swellings, distinct. (3-4 mm.)
4724	16	5		Slight swelling at threads. (2.5 mm.)
3703	16	6	4	Decided swellings at threads. (3.5 mm.)
4674	16	6	6	Slight enlargement.
58	5	11		Negative. Ovaries removed at operation.
61	4	11		Negative. Ovaries removed at operation.

Inasmuch as our operations upon the uteri of animals after stimulation of the cervical canal had demonstrated that the deciduomatous response is greater when the cycles are three or four days of age, we picked upon this as the earliest time at which the silk loops could be advantageously inserted in the uteri of lactating animals and expected that a long continued growth might be obtained of the deciduomata thus produced, since lactation serves to maintain for a month a constant condition of affairs in the reproductive tract. Three experiments were consequently done in this manner (Rats 4681, 69 and 59) in which

a wait of ten or eleven days took place before autopsy, but to our surprise no uterine tumors were then present. On the other hand, three similar experiments in which a wait of only six or eight days was enjoined gave large and histologically typical tumors (Rats 4680 [fig. 90, compare with fig. 91, pl. I], 4671 and 4670). It appeared probable that in the former three cases the deciduomata after having been produced had gradually degenerated. It was apparent that an irritant like the silk thread could provoke the production of these tumors when the uterus is in the proper "sensitive" state, but that these tumors had a limited span of life after their formation and that some other factors were necessary for their continued maintenance and growth.

It next appeared important to us to discover how late in lactation the uterus was "sensitive," providing due attention were paid to the fact that the four or five day interval after the threads were inserted is the ideal time to discover deciduomata in full vigor. Operations at six, seven, eight, and even ten days post-partum gave typical deciduomatous uterine responses when the animals were sacrificed four days after the operation (Rats 3637, 3591, 3620, 3623). Indeed, in one case when a wait of eight days was enjoined before autopsy, large typical tumors were still found (Rat 4678). Although the cases of operations conducted at a still later time during lactation are too few in number, we may state that operations even at the sixteenth day post-partum may succeed in producing a characeristic tumor (Rat 3730).

To summarize: The evidence presented in tables 27, 28, and 29, in spite of a number of individual discrepancies, shows that the experimental production of deciduomata in the rat might be viewed as dependent upon the presence of healthy, functional corpora lutea at least three days of age and that all conditions in which the life of corpora is prolonged serve also to prolong the time during which these tumors can be provoked by irritation of the uterine epithelium. Most of the discrepancies occurring in tables 27 and 28 are explainable by the appearance of oestrus before the experiment was terminated and by our clear demonstration that oestrus is inimical to growth, or even maintenance, of these tumors. *The evidence of the production of deciduomata is thus strictly consonant with the view that normal implantation is brought about through the aid of lutein cell hormones, hormones which are apparently produced only by corpora that have attained an age corresponding to the time interval normally occupied by the transit of fertilized ova through the Fallopian tube—in the rat an interval of about four days.*

4. EFFECT OF OVARIOTOMY

Corner and Warren in this laboratory have previously established the fact that after ovariotomy deciduomatous tumors can never be provoked by uterine irritation, and four cases studied by us (Rats 3447, 3548, 58, and 61) further confirm these findings.

VIII. EFFECT OF ABLATION AND TRANSPLANTATION OF VARIOUS PORTIONS OF THE REPRODUCTIVE TRACT UPON THE NORMAL OESTROUS CYCLE, PREGNANCY, AND LACTATION

A. Hysterectomy

Vague surmises have existed from time to time in the gynecological literature as to a supposed detrimental effect on the ovaries of complete removal of the uterus, the contention being occasionally made that the menopause is hastened by such a procedure. It consequently appeared to us of some importance to examine the effect on the oestrous cycle of complete ablation of both horns of the uterus. Inasmuch as in such animals as the rat and guinea pig the cyclic phenomena in the reproductive tract manifest themselves in the vagina as well as in the uterus, it should be possible to detect in such a mammal whether or not hysterectomy destroys this periodicity. Such evidence would be more difficult to obtain in man where cyclic changes in the vaginal fluid are not known independently of menstruation, which is occasioned by uterine hemorrhage. Accordingly, in vigorous young females possessing normal oestrous cycles the uterus was completely removed save for a small portion of the cervix. The vaginal smear was examined daily for several months thereafter, and in no instances, except for an occasional short pause immediately following the operation, was there any interference with the regular progress of normal oestrous cycles (table 30). These experiments would thus appear to demonstrate conclusively that hormones from the uterine tract are not concerned in the causation of the cyclic phenomena of oestrus, which, in the absence of this viscus, run their usual course and provoke their usual changes in the mucosa, even of the more external portion of the generative tract—the vagina. The typical effect of cervical stimulation in causing the delay of ovulation was also secured in these hysterectomized animals.

The importance of the cervical canal in the procreative act of this animal led us to desire to test, however, whether this tissue had a more important participation in oestrus than the uterus. Accordingly, in several cases we attempted a complete excision of the cervix and upper portion of the vagina along with both uterine horns. The operation is not easy, one difficulty being in the preservation of the ureters. At least a single entirely successful case of such an ablation was accomplished and the daily vaginal smear for many weeks thereafter showed the regular recurrence of normal oestrus.

Hysterectomy is equally innocuous in its effect upon lactation, a result which we might conceivably have been led to expect inasmuch as ovariotomy itself does not gravely influence lactation. Several cases of the combined excision of ovaries and uterus during lactation have given similar results, the litters evidently enjoying normal nutrition, to judge from their vigorous growth.

TABLE 30.

Length of œstrous cycles after hysterectomy.

Designation of animal	Length of cycle in days
3579	11, 4, 4, M14*, 4, 4, 4, 4, 4, M5, 4, M4, M16, 4, 11.
3599	14, 5, 4, 4, M19, 4, 4, 4, 4, M21, 5.
3820	11, 4, 4, 5, 6, M14, 4, 5, 5, 4, M13, M20, 12, 4, 7, 8, no more cycles (animal very thin).
4869	10, 4, 5, 5, 4, 5, 8, M8, 6, 4, 4, M4, M25, 4, 5.
4870	17, 6, 4, 4, M15, 4, 4, 4, 4, 4, M15, 4, 4, M14, 5.
4871	Died 10 days after operation; no cycles.
4872	16, 4, 5, 4, M15, 4, 5, 4, 4, 4, M16, 4, 4, M13, 4.
4873	13, 12, 17, 4, 4, 4, 4, 5, 5, M20, 6, M17.
4874	15, 6, 4, 4, M20, 5, 4, 4, M4, M18, 7, cervix removed, 8, 6, 12, 9, died.
4875	3, 5, 3, 5, 6, 4, 4, 4, M13, 5, 4, 5, 4, M17, 4, 4, M8, 5, 4.
4876	14, 8, 9, 6, 4, 6, 21, 4, 7, 7, 3, cervix removed, 4, died.
4878	Ovaries and uterus removed; suckled for at least 11 days.

*M cycle preceded by mechanical stimulation of cervix.

B. EXCISION OF MAMMARY GLANDS

On account of the extensive, irregular, and somewhat ill-defined tract occupied by the mammary glands of the adult, the total excision of the mammary apparatus in adults is difficult, if not impossible. Within the first eight days after birth the mammary anlagen are, however, confined to an area within one or two millimeters of the nipple and their extirpation can be easily carried out under the binocular microscope. The twelve small cutaneous defects created by the operation do not require suture, the young epidermis closing over with surprising speed. We experienced no difficulty in getting such young to be received again by the mother and to nurse normally. The animals mature as do normal ones, nor is the oestrous cycle of such animals in any way atypical (table 31). The participation of the mammary apparatus in the oestrous cycle, which has come to be a well established fact, would thus not appear to be an essential link in the chain of functional factors in oestrus. It is evident that the underlying cause for oestrus affects the mammary just as it does the uterine or vaginal tissue.

Nor can it be urged that these glands, characteristic of the mammalia, exert any essential effect during pregnancy, although profound changes in them occur during the latter event. Five individuals in which the mammary apparatus was completely ablated were allowed to mate, the resulting pregnancy being normal in duration and all other observable respects.

TABLE 31.

Maturity and œstrous cycles in rats after total excision of the mammary glands during infancy.

Designation of animal	Age at operation	Age at maturity	Length of successive cycles in days
3746	8 days	*78 days	8, 8, 5, 5, 5, 7, 7, †M13, 4, 4, M10, ♂12, ♂12, 4, 4, 4, 5, 6, 4, 4, 4, 4, 4, 4, 4, 4, 4, M12, 4, M11, M13, 4, 4, 4, 4, 4, M10, 4, M11, 4, 4, 4, 4, 4.
3747	8 days	*78 days	13, 6, 5, 6, 4, 7, 3mm. M10, 9, M10, 6, 6, M10, 4, ♂ preg. 22, 4, 5, 7, 4, 4, 4, 6, 4, 5, 4, 6, M12, 5, 5, M14, 4, 4, M12, M11, 4, M13, 4, 4, 4, 4.
3748	8 days	*79 days	30† before first cycle, 5, 4, 3 mm. M8, 3, M12, 5, 4, 11, 4, M15, ♂15, 5, 7, 7, 4, 5, 4, 4, 4, 4, 3, 4, 4, M13, 5.
3749	8 days	102 days	4, 4, 5, 4, 15, 14, 5, 4, 9, M4, 10, 4, 4, ♂ preg. 24, 3, 4, 4, 5, 4, 4, 4, 4, 8, 4, 5, 4, M11, 4, 5, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4.
3750	8 days	*78 days	12, 5, 5, 4, 5, 9, 8, 5, M8, 6, M4, 9, M3, ♂15, 4, 4, ♂13, 3, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, M16, 4, M5, 8, M3, M13, 3, 4, 4, M13, 4, 4, 5, M4, 4, 4, 4, 4.
3751	3 days	*73 days	12, 4, 5, 8, 6, 7, 3, FM15, 4, 4, FM11, 4, ♂preg. 22, 12, 5, 4, 5, 4, 5, 4, 4, 4, 4, 4, M14, 4, M5, ♂13, 4, ♂15, 5, M12, 5, 4, 4, 4, 4, 4, 4,5 .
3752	3 days	74 days	21, 7, 7, 16, 5, 11, no cycles Nov. 3 to Feb. 11, sickly.
3753	3 days	78 days	18, 13, M12, 5, 11, 4, 5, 4, M5, 4, ♂13, ♂13, ♂preg. and resorption, 35, 4, 4, 4, 4, 4, M4, M4, 5, M4, M4, 3, 5, 4, M4, 4, M4, M16, 5, 5, 4, M4, 4, 4, 4, 4, 4, 4.
3754	3 days	86 days	9 days before first cycle, 9, 6, 6, 11, M9, 5, 5, M5, 7, M5, 5, ♂12, ♂11, 5, 5, 7, 5, 4, 4, 4, 4, 4, 4, 4, 4, M6, 4, 4, 4, M14, 8, 8, 10, 5, 11, 8, 7, 4, 4, 4.
3755	3 days	94 days	8, 10, 8, M4, M5, 6, 6, 4, 6, 5, FM14, ♂15, 4, 5, 5, 9, 5, 5, 4, 4, 4, 5, 3, 5, 3, 4, 5, 8, 4, M4, 4, 9, 8, 9, 5, 5, 4, 5, 5, 5, 4, 12.
3756	3 days	77 days	18, 6, 12, 15, 12, 7, FM5, 4, FM5, 4, 4, ♂preg. 23, 14, 11, 5, 5, 4, 4, 4, 8, 4, 4, 4, M13, 3, 6, 10, 13, 4, 4, 4, 5, 4, 5, 4, 4, 4, 4.

*Age when observations were begun—vagina open.
M. Indicates mechanical stimulation of cervix by insertion of a rod; figure following, the length of next cycle.
FM. Failure to introduce rod into cervix.
3mm.M. Rod introduced only 3 mm. In other instances inserted about 10 mm.
♂ Copulation with normal male.

C. Ovariotomy

We can confirm the experience, which is universal, that excision of the gonads brings about a sudden and permanent cessation of all cyclic changes in the reproductive tract. A double ovariotomy was carried out upon twenty-five rats at various times in the normal oestrous cycle. In some cases, notably those in which the operation was carried out immediately preceding an expected oestrus, the next expected typical oestrous changes in the vaginal smear occurred so that it is evident that the impulse or cause of oestrus operates slightly anterior to the actual event. In all cases of ovariotomy the vaginal smear showed henceforth no indications whatever of an oestrous cycle, daily observations having been carried out for some months subsequent to the operation. The effect of extirpation of the gonads upon the structure of the uterus and vagina, where a gradual atrophy is encountered, has already been reported by Marshall.

We are able to confirm the discovery that ovarian ablation interferes in no respect with the continuance of lactation, vigorous litters having been reared after such ovariotomies (Rats 53 and 54).

Previous observers (Fränkel, 1903, for the rabbit; Marshall and Jolly, 1904, for the rabbit and rat) have reported the necessity of the ovary (on account of its possession of lutein tissue) for the implantation and nutrition of the early embryo, but have indicated that the ovaries may be ablated in the latter half of the pregnancy with impunity.

D. Ovarian Transplantation

The detection of the oestrous cycle in the rat by means of the vaginal smear and the fact that oestrus never recurs after ovariotomy, furnishes us with an ideal test for the success of ovarian transplants. We have carried out transplantation of the ovaries in twenty-nine cases, the site of the graft being in the mesometrium, omentum, spleen, or rectus muscles. In all cases vigorous young females were employed. Nineteen of the cases were instances of autotransplantation (table 32) where the expectation of success is, of course, greatest, and we obtained fourteen favorable results here as against five failures. Ten cases were attempted of homotransplantations (table 33) and in such operations both the donor and recipient were prepared simultaneously so that the exchanges were effected in the shortest possible time. It is to be noted that only one entirely successful outcome attended these efforts, seven of the cases having given at no time indications of the success of the graft and two of them a single oestrous cycle only. In the two cases in point, the single post-operative oestrus produced occurred so late (seven days) that we are not to attribute this

to the spontaneous occurrence of the next expected oestrus, as sometimes happens after ovariotomy, but must admit an ephemeral "take" of the graft with secondary and speedy resorption. Doubtless more success would attend homotransplants which were between mother and daughter possessing the same male parent. In all instances the ovary to be transplanted was cut into two or more pieces to facilitate vascularization before decay, which in the case of large pieces of tissue may be extensive.

Daily observations of the vaginal smear have been carried out for somewhat over seven months subsequent to these operations. They demonstrate that the successful ovarian grafts produce the next oestrous cycle as a rule one week after the operation, this varying in the time of its occurrence from five to twelve days after the transplants were made. In practically all the successful cases throughout this long time interval (seven months) a regular succession of typical oestrous cycles of normal length has occurred. When observations were discontinued the animals were almost one year of age, which approaches the end of the normal period of full reproductive vigor for this species. Our experiments thus do not lend any support to the assumption that the life of the ovarian transplants need be shorter than that enjoyed by the gland in its normal situation.

The oestrous changes experienced by animals with transplanted ovaries were, as far as we could determine, entirely normal. In order to discover whether cyclic changes in the transplanted ovaries corresponded with oestrous stages in the vagina we examined the transplants of several typical successful cases at the stage in the vaginal smear when leucocytes first appear among the cornified epithelial cells, a stage in which under normal conditions a fresh ovulation should have occurred. In such instances the transplanted ovary (fig. 96, pl. IX) disclosed a group of new corpora together with other corpora from the immediately preceding ovulations at four-day intervals. A normal content of healthy follicles and of follicular atresia was also present in these ovaries so that study of them did not indicate any departure from the normal phenomena found in this gland. The transplanted ovaries evidently mature follicles which rupture at regular intervals and are transformed into corpora. Under the circumstances the ova are evidently expelled into the tissue immediately surrounding the transplant rather than, as normally, into the fluid-filled periovarian space. They evidently speedily degenerate, for in two instances where the ovulation must have been recent no traces of eggs were discovered. Difficulty in the expulsion of the ovum under these conditions might explain the occurrence of several cases of the retained egg about which the corpora have formed, an occurrence by no means absent in the normal ovary, as we have already shown, but where the proportion of such cases is, of course, somewhat lower than we would expect in these transplanted cases.

TABLE 32.

Effect of ovarian autotransplantations upon the œstrous cycle as determined by vaginal smears.

Designation of animal	Position of transplant	Interval between operation and first cycle in days	Length of cycles in days
3883	Spleen	10 days	8, 6, 5, M13, 19, 11, 10, 3, 5, 2, 5, 4, 6, M17, 6, M15, 4, M4, M14, 5, 4, M4, 8, 6, 5, 5.
3885	Spleen		No cycles.
3886	Spleen	8 days	5, M4, 4, M4, 4, 17, 5, 4, 8, 3, 5, 7, 3, 5, 5, 4, 8.
3887	Spleen	12 days	8, 8, 3, 3, 4, 8, 4, 4, 6, 4, 4, 6, 4, 4, 8, 4, 4, 7, M13, M13, 4, M6, 7, 11, 15, 7.
3888	Spleen	7 days	5, 5, M6, 4, 5, 5, 6, 4, 7, 5, 4, 5, 6, 6, 4, 4, 4, 5, 6, 6, 6, M20, 5, 3, M4.
3889	Spleen		No cycles.
3890	Spleen	5 days	5, ♂4, 4, 5, 6, M4, M12, 4, 4, 4, 4, 4, 4, 4, 3, 4, 5, 4, 5, 4, 4, 4, M4.
3891	Spleen		No cycles.
3892	Spleen		No cycles.
3893	Spleen	7 days	6, 4, M♂4, M9, 16, 4, 4, 4, 5, 5, 6, 4, 4, 4, 4, 4, 4, 7, M18, 5, 5, M15, 5, M17, 5, 5, 4, 6, 13, 5.
3894	Spleen		No cycles.
3895	Spleen	12 days	9, 5, 6, M10, 10, 43 days without cycles.
3896	Rectus muscle	7 days	6, M3, M and ♂ 3, M4, M12, M16, 5, 5, 5, 11, 4, 4, 3, 4, 4, 4, 4, 4, 4, M18, 4, M20, 9, 5, M18, 5, 5, 5.
3898	Rectus muscle (both ovaries)	6 days	16, 4, 4, M17, 4, 4, 4, 4, 5, 4, ♂16, 4, 4, 4, 4, M23, M16, 4, 3, 4, M22, 4, M19, 5.
3899	Rectus muscle (both ovaries)	6 days	5, 5, M14, M4, M4, 4, 4, 4, 4, 7, 4, 4, 4, ♂19, 4, 5, 5, M5, M10, 4, M19, 5, M6, M4, 6, 7, 4, 4, 10, 12.
3900	Mesometrium (both ovaries)	5 days	4, 4, M♂3, 10, 3, M20, 4, 4, 3, 5, 4, 4, ♂18, 4, 4, 4, M16, 4, M14, 4, M13, 4, 3, 5, 5, 3, M16, 4, 4, 4, 3.
3901	Recti musculi (both ovaries)	7 days	5, 4, M4, 5, 4, M4, M14, 3, 5, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, M4, M6, M23, 4, M4, M20, 4, 4, 5, 5, 4, 4, 3, 3, 4.
3902	Both recti (both ovaries)	9 days	6, 14, 5, Killed.
3903	Both recti (both ovaries)	7 days	5, 5, 6, 2, 4, 4, Killed.

19 instances: 14 successful; 5 failures.

M. Indicates mechanical stimulation of cervix by insertion of a rod; figure following the length of next cycle.

♂ Copulation with normal male.

TABLE 33.

Effect of ovarian homotransplantations upon the œstrous cycle as determined by vaginal smears.

Designation of host	Designation of donor	Position of transplant	Interval between operation and first cycle in days	Length of cycle in days
3535	3884	Spleen		No cycles.
3561	3883	Spleen	7 days	No cycles.
3603	3885	Spleen		No cycles.
3610	3890	Omentum		6, no further cycles.
3614	3891	Spleen		No cycles.
3618	3889	Spleen		No cycles.
3620	3893	Spleen		No cycles.
3625	3887	Spleen		No cycles.
3627	3894	Spleen		No cycles.
3653	3888	Spleen	7 days	6, 5, M5, 3, 6, M24, 4, 7, 13, 5, 5, 3, 4, 7, 5, M9, M5, M6, 10, M5, 13, 5, 5, M5, 15, 3, 18.

Successful ovarian transplantation afforded us an ideal opportunity to discover whether the effect of mechanical stimulation of the cervical canal in delaying the next oestrous took place through mediation of the nervous connections of the ovary. We may state at once that delay in the appearance of the next oestrous cycle after coitus with vasectomized males or after cervical stimulation was obtained in all animals with transplanted ovaries, and it would seem probable that this effect on the oestrous cycle and especially upon the performance of the ovary is not invoked by the employment of nervous pathways. It is presumably not entirely out of the question that new nerve connections established by the transplant could mediate this result, but several instances were obtained of the effect of this procedure so early in the life of the young transplant that it is highly unlikely, if not impossible, that the perfection of a new nervous arc had resulted. Our experiments would hence indicate that the ovarian nervous system is not concerned in this astonishing reaction. Nevertheless, it is to be admitted that if a hormonal explanation is resorted to, it is difficult to believe in the absorption of the minute amount of substances created by mechanical contact with slight injury of the superficial cervical mucosal cells, an absorption which must take place through the high stratified, squamous epithelium there present.[5]

[5] Since the above was written M. F. Fryer has injected suspensions of injured epithelial cells from the lower portion of the cervical canal of oestrous donors into the body cavity of normal recipients without effect on the cycle of the latter.

E. Exchange of Ovaries Between Young and Old Animals

The dependence of the phenomenon of oestrus upon an actively functioning ovary, which all the above experiments have demonstrated, and especially the fact that the first ovulation is often coincident with and never occurs before the establishment of the vaginal orifice led us to inquire to what extent we could hasten the development of the generative tract or whether we could induce it to undergo precocious oestrous cycles by an exchange of ovaries between mature and immature animals. Accordingly, seven instances (table 34) of such exchanges were carried out, the ovaries being in each case transplanted to the rectus muscles. The immature rats were vigorous young females from twenty-two to twenty-nine days old, the adults being about five months of age and in complete reproductive vigor. In most instances, in spite of the fact that a functionally mature gland was now in contact with youthful tissue, no hastening of the establishment of the vaginal aperture resulted, nor after the formation of the latter were oestrous changes ever observed in the vaginal smear. The adult transplants must have speedily succumbed. The experiments demonstrate merely that when ovarian extirpation is carried out after the twenty-first day of life sufficient further development of the reproductive tract to involve breakdown of the vaginal membrane takes place. Since the transplantation of ovaries which are older or younger than the tissues of the host must be a homotransplantation, it is possible that protein incompatibility led to failure of the "take" of these grafts, and that we are not necessarily justified in stating that *successful* grafts of old ovaries upon young females would not hasten the advent of the first oestrus.

Table 34.

Effect of the exchange of ovaries between rats 22 to 29 days old and adults. Transplantations were made into the rectus muscle.

Designation of animal	Age of rat in days at operation	Age of ovary	Age of rat in days when vagina opened	Length of cycle in days
4039	141	22 days	....	7. No more cycles.
4048	167	24 days	....	6. No more cycles.
4050	172	22 days	....	8. No more cycles.
4107	167	29 days	....	6, 4; killed. Ovaries sectioned.
4109	165	29 days	....	7, 9. No more cycles.
4205	128	29 days	....	6, 10. No more cycles.
4221	126	22 days	....	6. No more cycles.
4654	29	167 days	79	No cycles.
4656	22	126 days	97	No cycles.
4657	22	172 days	79	No cycles.
4658	22	141 days	79	From opening to first cycle: 3 days. Cycles: 9, 4, 10.
4661	29	128 days	....	Not open at 113 days.
4662	29	165 days	92	No cycles.
4663	29	167 days	75	No cycles.

On the other hand, the behavior of the immature ovaries placed within the musculi recti of adults was highly interesting. *These minute glands, whose approximate dimensions did not exceed two millimeters, grew rapidly and in every instance in from six to eight days brought on typical oestrus of the host.* Studies of such ovaries showed that the oestrus experienced by the host was associated with the same sequence of events in the young transplants as characterizes the adult gland at such a time, i.e., the enlargement and bursting of follicles and the production of typical corpora lutea. In all of these cases at least a single oestrus was thus produced by the young graft. In four instances no more oestrous cycles were produced in the adult host, for the infantile ovarian graft then apparently promptly atrophied, but in three instances another oestrous cycle followed the first, in one case at the normal four day interval. On the appearance of leucocytes among the cornified cells in the vaginal smear of this case we sacrificed the animal to convince ourselves of the performance of ovulation by the youthful gland. Five young corpora lutea were found, three of them being normal ones. In two cases the ovum was retained within the follicle, but the granulosa cells underwent typical lutein cell transformation. The three other follicles evidently discharged their ova, for the central cavities of these three corpora did not contain the eggs. In the other two instances of the occurrence of two oestrous cycles no further cycles took place, the ovaries in these cases evidently undergoing the same atrophy and resorption which characterized all the other cases of youthful ovaries in adult tissues. It is clear that the adult environment had succeeded within seven days in bringing the minute ovaries of animals less than a month in age to a condition of functional maturity, usually only attained by animals ninety days in age and never attained before the forty-fifth day of post-natal life.[6]

It would appear likely that the final atrophy of the young transplant must be due to the same causes which determine a similar fate of the grafts of adult ovaries into young animals, i.e., protein incompatibility, and it is possible that in both cases a preliminary "take" of the grafts occurred with secondary re sorption. This short interval of life experienced by the homografts was sufficient in the case of the adult hosts to mature the youthful grafts, but in the case of the young hosts was not sufficiently long to bring to conclusion the development of the vaginal canal and especially the breakdown of its closing membrane. The experiment would appear to afford an elegant demonstration of the effect of hormones from the soma upon the time of maturation of the germ cells.

[6] Since the above was written one of us has encountered two instances of sexual maturity on the thirty-fifth day of life in a group of about six hundred females.

IX. SUMMARY

1. The length of the oestrous cycle in the rat is about four days.

2. It is characterized by regular, periodic, coördinated, histological changes in every portion of the reproductive tract. These changes manifest themselves especially in the growth, degeneration, and regeneration of the epithelium of the uterus and vagina, and in the growth and rupture of ovarian follicles with consequent formation of corpora lutea of ovulation.

3. The above phenomena are coördinated with oestrus and ovulation, relations evidently essential for the fertilization of ova and implying a limited viability on the part of both male and female sexual elements subsequent to their discharge.

4. These changes may be followed in the vagina in the living animal by the microscopic examination of its contents.

5. Five different histological pictures are presented by the vaginal smear, and may be used as criteria for stages in the cycle.

6. *Stage One.* Smear of uniform-sized epithelial cells only.

Vagina: Lips somewhat swollen, mucosa slightly dry, 8 to 12 layers thick, stratum corneum appearing beneath surface layer, no leucocytes.

Uterus: Becomes distended with fluid toward end of stage.

Ovary: Growth and enlargement of follicles; corpora lutea of preceding ovulation now show fatty degenerative changes.

Heat is manifested toward the end of the stage.

Average length, twelve hours.

Stage Two. Smear of cornified cells only.

Vagina: Lips swollen, mucosa dry and lusterless, cornified layer now superficial and beginning to dehisce, no leucocytes.

Uterus: Reaches greatest distention in early part of stage and then regresses, vacuolar degeneration of epithelium sometimes begins.

Ovary: Large Graafian follicles, eggs may undergo maturation.

Heat is manifested during most of stage.

Average length perhaps twelve hours.

Stage Three. Smear of large numbers of cornified cells only.

Vagina: Lips sometimes still swollen, mucosa dry and lusterless, cheesy substance in lumen, cornified layer completely detached, no leucocytes.

Uterus: Epithelium undergoing vacuolar degeneration.
Ovary: Ovulation, secretion of fluid into periovarial space and distal folds of oviduct.
Animal not in heat.
Average total length of stages two and three, twenty-seven hours.

Stage Four. Smear of cornified cells and leucocytes.
Vagina: Swelling of lips gone, mucosa slightly moist, greatly reduced in height, infiltrated with leucocytes.
Uterus: Vacuolar degeneration reaches its height and regeneration proceeds *pari passu.*
Ovary: Young corpora lutea containing cavity or with cavity just closed; follicles smallest, eggs in oviduct.
Average length, six hours.

Stage Five. Smear of leucocytes and epithelial cells.
Vagina: Mucosa moist and glistening; thin; some leucocytes.
Uterus: Epithelium regenerated.
Ovary: Corpora lutea normally fully formed and in vigorous function.
Average length, fifty-seven hours.

7. Essentially the same succession of oestrous changes occur in both rat and guinea pig, although in the latter the cycle is much longer.

8. About ten corpora are formed per ovulation, one of which retains the egg.

9. The average number of young per litter is seven.

10. About one-third of the eggs reaching the oviduct fail to produce young.

11. The use of vital dyes furnishes the only reliable method of identifying corpora.

12. There are four kinds of corpora lutea, those of ovulation, copulation, pregnancy, and lactation, in accordance with the sexual history. Corpora formed immediately after parturition may become any one of the above four types.

13. The first three types are alike in number, size, and distribution of lipoid granules; the corpora of lactation differ in having smaller granules.

14. A characteristic increase in the size of these granules takes place either before or by the beginning of the next oestrous cycle.

15. In our opinion these lipoid changes mark the end of the period of functional activity of the corpus.

16. The formation of the vaginal orifice is usually coincident with the first oestrus and ovulation, and in other cases takes place on the average about four days earlier; exceptionally from ten to thirty days earlier.

17. The first oestrus in a certain lot of animals handled by us occurred on the average on the seventy-sixth day, but it may occur much earlier.

18. Most instances of the first copulation occur about ten days later.

19. The first oestrous cycles are longer than the normal, the first averaging ten or eleven days.

20. Copulation itself delays the appearance of the next oestrus, and produces conditions in the vagina and ovary which may appropriately be termed pseudo-pregnancy.

21. Identical effects are secured by mechanical stimulation of the mucosa of the canal at the tip of the cervix.

22. Copulation hence produces its effect through the extension of part of the plug into the cervical canals.

23. This effect of copulation in the rat may be interpreted as a means of insuring implantation by delaying oestrus.

24. The average length of gestation is slightly under twenty-two days.

25. Characteristic changes in the vaginal mucosa are produced by pregnancy and by lactation.

26. In pregnancy the vaginal epithelium increases in height, the cells of its intermediate layers undergo vacuolization, and its surface cells are columnar.

27. Stimulation of the cervix produces similar changes in the vaginal epithelium.

28. In lactation the vaginal epithelium is depleted to two or three cell layers, the superficial ones being columnar.

29. Ovulation does not occur during either pregnancy or lactation. Inhibition of ovulation in the early part of pregnancy is due to copulation (cervical stimulation), but in the latter half must be due to foetal hormones.

30. Stimulation of the uterine mucosa does not affect the oestrous cycle, but under certain conditions produces deciduomata.

31. Deciduomata can not be produced during normal oestrous cycles.

32. They may be produced during a lengthened oestrous cycle caused by stimulation of the cervix, and during lactation.

33. The earliest time at which they were produced was the fourth day after oestrus.

34. This approximately coincides with the time when blastodermic vesicles normally reach the uterus.

35. Deciduomata are resorbed with the onset of the next oestrus.

36. It is suggested that implantation would fail if copulation did not inhibit the next oestrus.

37. The oestrous cycle is normal in the absence of the uterus, the uterus and cervix, or the mammary glands.

38. Stimulation of the cervix produces its typical effect after excision of the uterus or mammary glands.

39. The cycle ceases after ovariotomy.

40. Successful transplantation of the ovary is demonstrated by the return of typical cycles.

41. Transplanted ovaries rhythmically mature follicles and produce corpora.

42. Stimulation of the cervix produces its typical effects in animals with transplanted ovaries.

43. Ovaries of rats 21 days old transplanted to adult rats mature in about a week, an acceleration of about two months.

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EXPLANATION OF PLATES

List of abbreviations used on plates:

l. c., lutein cell.
ov., corpus luteum of ovulation.
1 ov., corpus luteum of first ovulation.
2 ov., corpus luteum of second ovulation.
cop., corpus luteum of copulation.
pr., corpus luteum of pregnancy.
lac., corpus luteum of lactation.
y.c., young corpus luteum still containing central cavity.
f., follicle.

Figures 61 and 62 have been omitted.

PLATE I

Fig. 8. Ventral view of rat laid open to show the slender uterus characteristic of the interval. Large quantities of fat are attached to the mesometria. × ⅔.

Fig. 2. View of the vaginal portion of the cervix to show the lappets about the orifices of the cervical canals. Hairs mark the openings of the canals. × ca. 7.

Fig. 3. Aspect of the external orifice of the vagina with the clitoris above and anus below, to show in "a" the condition during the interval, and in "b" the greater prominence of the opening at oestrus caused by the turgescence of the folds about the orifice. × ca. 1.5.

Fig. 90. Horn of uterus and ovary of rat to show two placentomata produced by the insertion of threads in uterus on the 4th day of lactation; rat killed 6 days later. The upper thread merely passing around the uterus had no effect. Compare with normal 9 day pregnancy in figure 91. Rat 4680. × ca. 1.5.

Fig. 91. Portion of one horn of uterus of rat killed on the 9th day after copulation showing two implantation sites. Rat 3431. × ca. 1.5.

Fig. 15. Ventral view of abdominal cavity laid open to show the uterus late in Stage One; the uterus is greatly distended with a characteristic clear fluid. Compare with figure 8. × ⅔. The uterus reaches its greatest distention at the end of Stage One and the beginning of Stage Two.

Fig. 41. Photograph of section through the folds of an oviduct to show an egg in the distended distal portion of the wall, which has ridges covered with cilia, and ovary which contains a young corpus with large cavity. The vaginal smear was observed at 3 hour intervals for 8 days. The animal was killed 21 hours after the beginning of Stage Two. Rat 4238. Table 2. × 20.

Fig. 40. Photograph of section of oviduct of rat killed just before ovulation. The distal fold marked by the presence of ridges has a greatly restricted lumen. The vaginal smear was observed at 3 hour intervals for 27 hours. The animal was killed in Stage Three, 18 hours after the beginning of Stage Two. Rat 4081. Table 2. × 20.

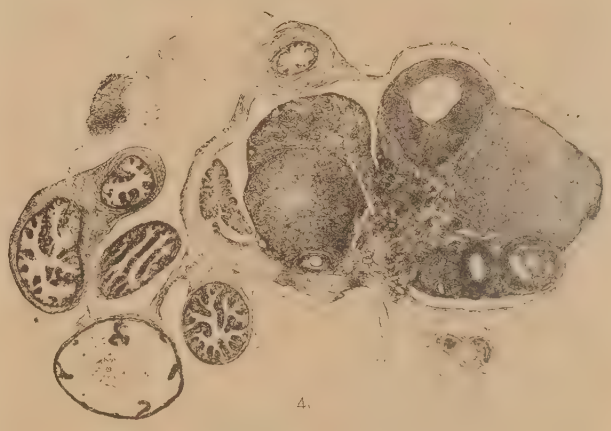
8

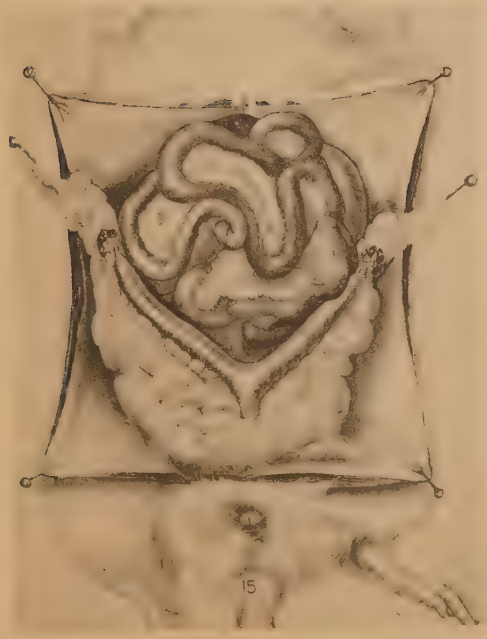
2

90

a b
3

91


4


15

40

PLATE II

Fig. 4. Photograph of a fresh smear taken during the interval to show the characteristic appearance under low magnification (× 67). Among the leucocytes one sees very faintly the epithelial cells.

Fig. 10. Low power photograph (× 67) of a fresh vaginal smear taken at the beginning of Stage One. It consists only of epithelial cells.

Fig. 21. Photograph of smear of fresh Stage Two to give appearance as seen at low magnification (× 67). The elements consist entirely of flakelike, cornified cells which have lost their nuclei. Note contrast with smear of Stage One in figure 10 and with interval in figure 5.

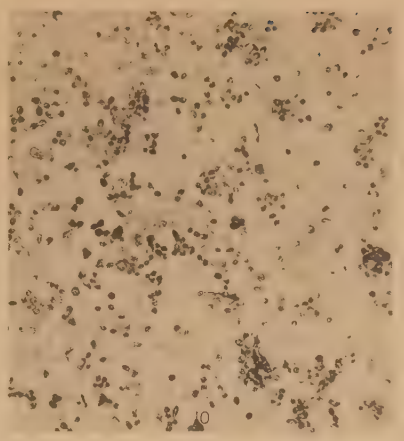
Fig. 31. Photograph of fresh smear in Stage Four to show appearance at low magnification. The smear consists solely of cornified cells and leucocytes. × 6.

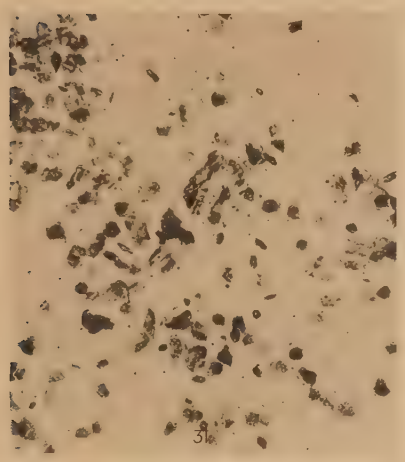
Fig. 32. Drawing of smear of Stage Four. Cornified cells and leucocytes. × 813.

Fig. 5. Polymorphonuclear leucocytes and epithelial cells characteristic of the smear made from the contents of the vagina during the interval or dioestrum. They are derived from an epithelium like those in figures 6 and 7, plate III. × 813.

Fig. 22. Five of the cornified elements of the smear from the vagina in Stage Two. × 813.

Fig. 11. Epithelial cells constituting smear of early Stage One. These cells are derived from the superficial epithelial cell layer of figures 12 to 14, plate III. × 813.





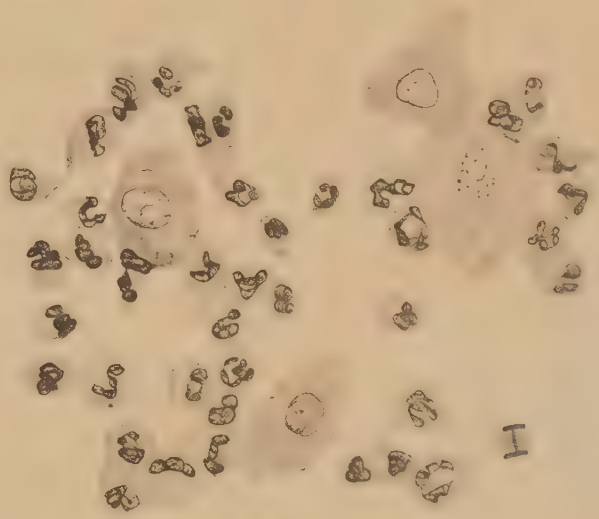


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PLATE III

Fig. 6. Vaginal mucosa from the middle of the doestrous interval. The epithelium contains leucocytes, and is at the lowest height of any time during a normal cycle. The vaginal smear was observed twice daily for 70 days; the last five cycles were of five days duration; the animal was killed about 30 hours after the beginning of the last dioestrum. Rat 3498. × 813.

Fig. 7. Vaginal mucosa very late in the dioestrous interval. The epithelium is thicker and shows the beginning of stratification more clearly. The vaginal smear was observed twice daily for 40 days; the cycles were of 4 days duration; the animal was killed in the dioestrum 24 hours before the next expected oestrus. Rat 2263. × 813.

Fig. 12. Vaginal mucosa of earliest part of Stage One. True cornification has not yet appeared, but the cells of the middle layers are becoming flattened. The most superficial cells remain full, show a little swelling, and become the superficial epithelial cells which are shed as the cells of the smear of Stage One. The vaginal smear was observed at 3 hour intervals for 8 days; the animal was killed 4 hours after the beginning of Stage One. Rat 3703. × 813.

Fig. 13. The same as figure 12, but evidently a little later. The mucosa is thicker, a stratum granulosum is present, and just *beneath* the superficial cell layer, cornification is beginning. The vaginal smear was observed from two to four times daily for 6 days; the animal was killed on the appearance of Stage One. Rat 2856. × 813.

Fig. 14. The same as figures 12 and 13, but still later. Cornification is still more advanced and the superficial cell layer is becoming detached to form the cells of the vaginal smear. At this time the mucosa reaches its greatest height. The vaginal smear was observed at 3 hour intervals for 8 days; the animal was killed 4 hours after the beginning of Stage One. Rat 3712. × 813.

Fig. 23. Vaginal mucosa in early Stage Two. The superficial epithelium is completely gone, leaving the thick cornified layer bare. The stratum granulosum is well marked, apparently indicating continued cornification. The flaking off of the cornified layer supplies the material of the smear shown in figures 21 and 22, plate II. The vaginad smear was observed at 3 hour intervals for 8 days; the animal was killed 2 hours after the beginning of Stage Two. Rat 3683. × 813.

Fig. 24. Vaginal mucosa late in Stage Two. Practically all of the cornified layer has been lost. The whole mucosa is thinner; a few leucocytes reappearing. The vaginal smear was observed at 3 hour intervals for 8 days; the animal was killed 54 hours after the beginning of Stage Two. Rat 3675. × 813.

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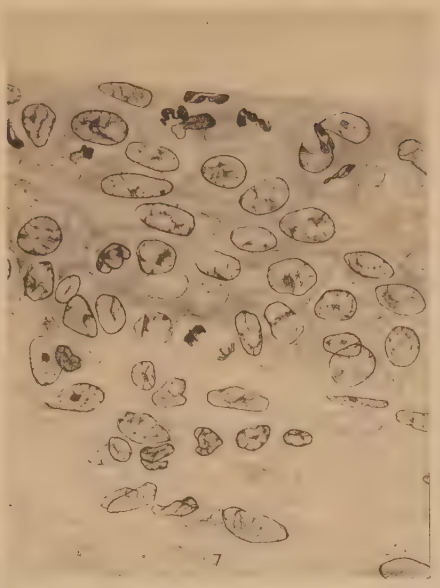
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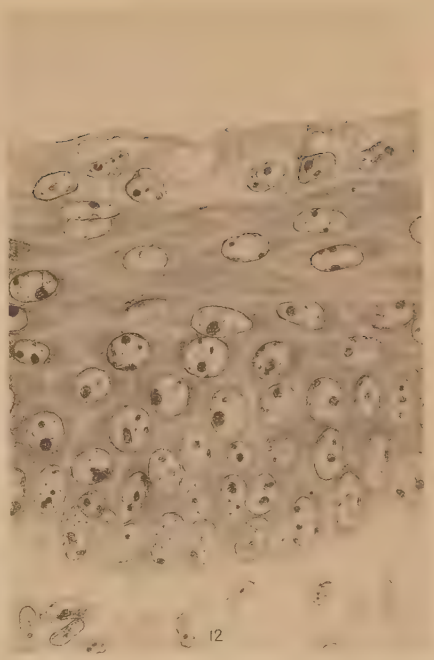
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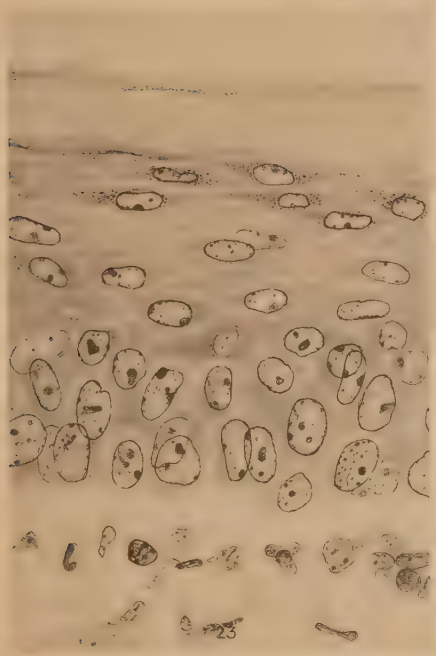

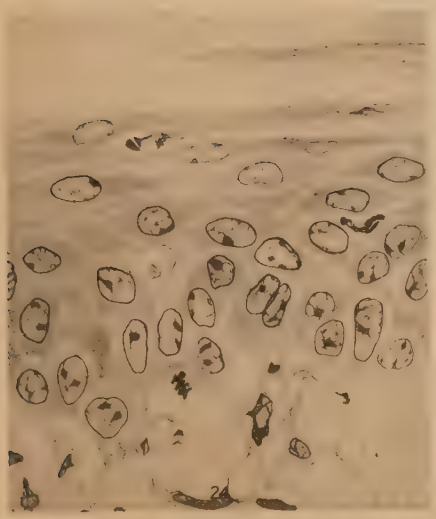
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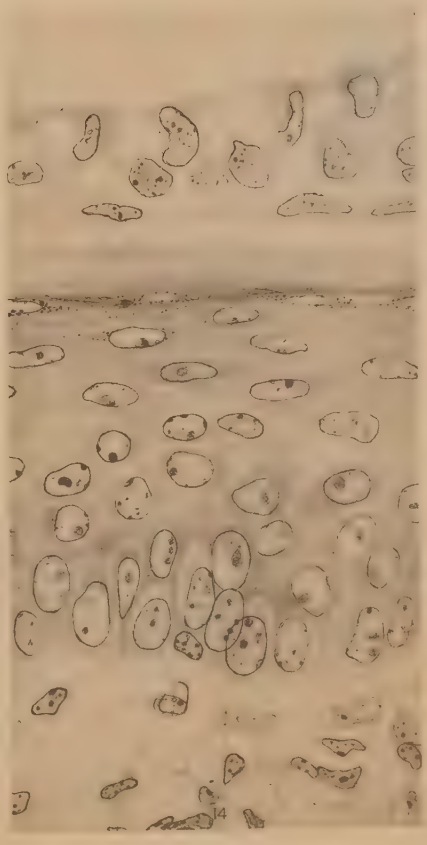
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PLATE IV

Fig. 28. Vaginal mucosa in Stage Three. The cornified layer is completely stripped to form the "cheesy" substance of the smear, the elements of which are the same as those in figures 21 and 22, plate II. The superficial flattened layers are less conspicuous. Leucocytes have reappeared. The vaginal smear was observed at 3 hour intervals for 8 days. The animal was killed in the cheesy stage, 48 hours after the beginning of Stage Two. Rat 3685. × 813.

Fig. 33. Vaginal mucosa early in Stage Four. The vaginal smear was observed at 3 hour intervals for 8 days. The animal was killed within two hours after the beginning of Stage Four. Rat 3647. × 813.

Fig. 34. Vaginal mucosa at end of Stage Four. At this stage there is no vestige of the stratum corneum or granulosum. The epithelium is infiltrated with leucocytes. The vaginal smear was observed at 3 hour intervals for 8 days. The animal was killed 5 hours after the end of Stage Four. Rat 3672. × 813.

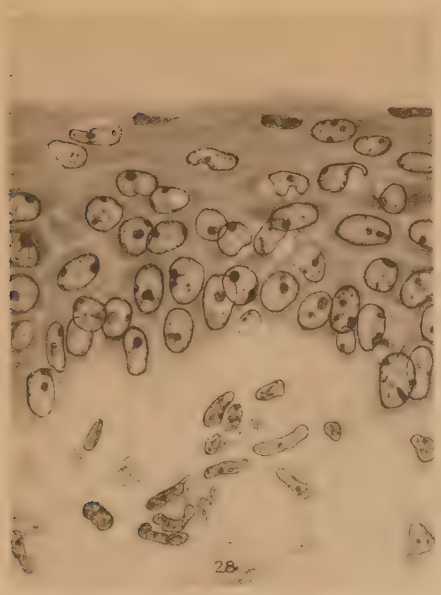
Fig. 63. Vaginal mucosa, 8th day of pregnancy. The superficial cells are low columnar in form. Rat 3501. × 813.

Fig. 64. Vaginal mucosa, 10th day of pregnancy. The superficial cells are typical columnar ones. Note the beginning of vacuolization of the middle cell layers. Rat 3558. × 813.

Fig. 65. Vaginal mucosa, 14th day of pregnancy. Selected to show a portion of the film of red corpuscles appearing suddenly about the 14th day of pregnancy. Does not show the vacuolization evident in other parts of the section. Rat 3473. × 813.

Figs. 66 and 67. Vaginal mucosa of 16th and 20th days of pregnancy respectively. The vacuolization of the intermediate cells has reached a maximum, and the columnar character of the superficial cells detectable in figures 63 to 65 remains evident. Rats 3471 and 3508. × 813.

Fig. 74. Vaginal mucosa on the 2d day of suckling. Compare with figures 75 and 76, plate V. Rat 3622. × 813.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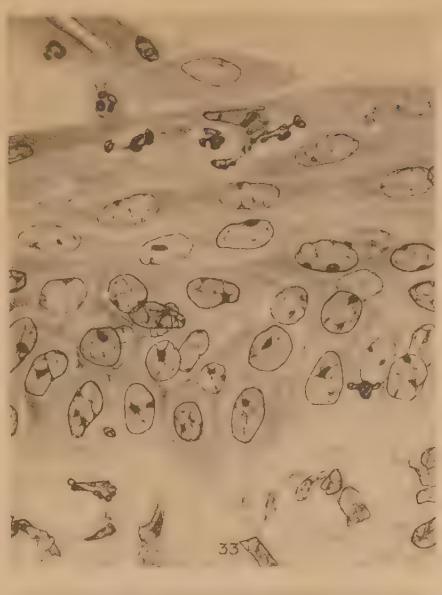
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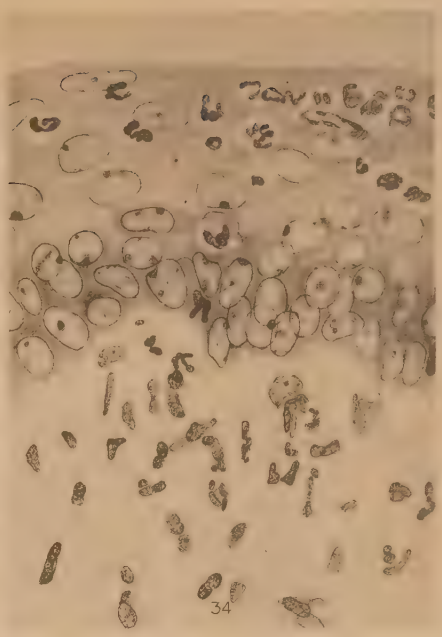
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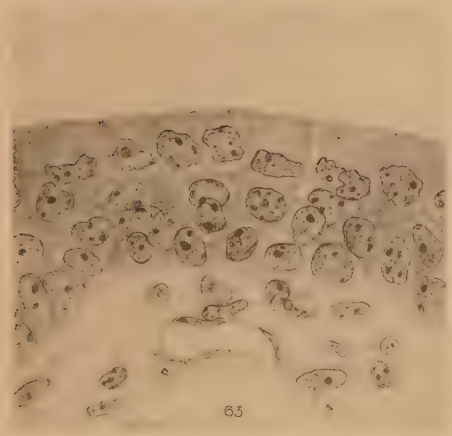
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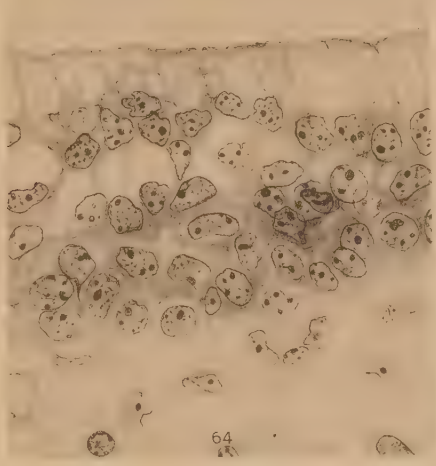
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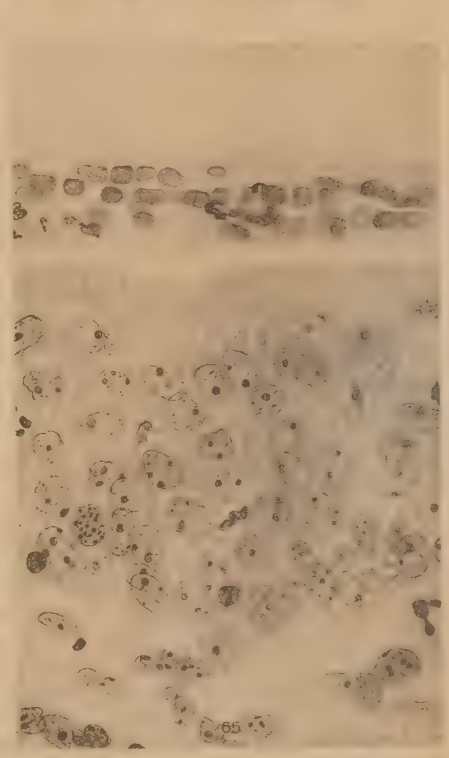
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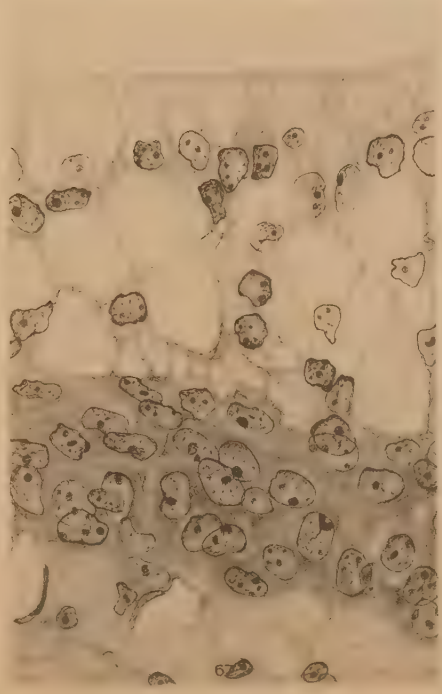
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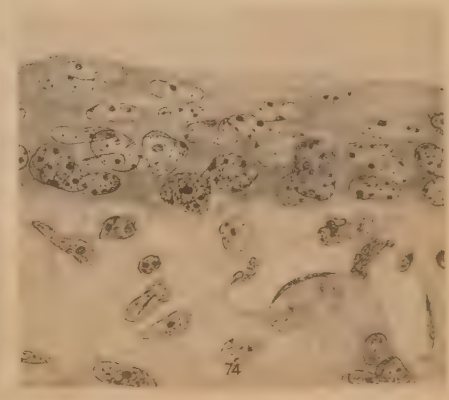
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PLATE V

Figs. 75 and 76. Vaginal mucosae on the 4th and 16th days of suckling respectively. Note the decrease in height during the first few days and the columnar nature of the superficial layer. (See fig. 74, pl. IV.) Rats 3635 and 3643. × 813.

Fig. 20. Uterine epithelium, about the middle of Stage One. Diameter of uterus about two millimeters. Rat 3712 (see fig. 14, pl. III). × 813.

Fig. 26. Uterine epithelium at time of greatest distention. See figure 25, plate VIII, Rat 2454. × 813.

Fig. 9. Uterine epithelium at the middle of the interval. (Rat 3498. See also fig. 6, pl. III.)

Fig. 30. Uterine epithelium in Stage Three, in a more advanced state of vacuolar degeneration. Rat 3685. (See fig. 28, pl. IV.) × 813.

Fig. 37. Uterine epithelium at the end of Stage Four. Although the vaginal condition indicates Stage Four (see fig. 34, pl. IV), ovulation has not occurred, and vacuolar degeneration has not progressed so far as in rat 3647 (fig. 36), which has eggs in oviduct. Rat 3672. × 813.

Fig. 27. Uterine epithelium after return of uterus to ordinary size. The epithelium is beginning to undergo vacuolar degeneration. Compare with later stages of degeneration in figures 30 and 36. Rat 3675. (See fig. 24, pl. III.) × 813.

Fig. 78. Uterine epithelium of rat on 16th day of lactation. Very clear columnar cells with dense outer portions. Rat 3643. × 813.

Fig. 86. Vaginal mucosa on the 13th day after stimulation of the cervix by means of a glass rod. Note the similarity to mucosa of pregnancy, figures 63 to 67, plate IV. Rat 3578. × 813.

Fig. 36. Uterine epithelium early in Stage Four. An advanced stage of vacuolar degeneration. Rat 3647. (See fig. 33, pl. IV.) × 813.

Fig. 77. Vaginal mucosa of rat 2 days after weaning. The mucosa has quickly increased greatly in thickness and the superficial epithelial cell layer has formed. Compare with figure 12, plate III. Given vital dye during pregnancy. Allowed to suckle litter of 10 for 6 days, when young removed. Killed 2 days later. Rat 3629. × 813.

PLATE VI

Figs. 55 to 58. Paintings of ovaries of rats given Dianil Blue 2R during pregnancy and killed at parturition, and at 4, 9, and 51 days after parturition respectively. After littering the animals were not allowed to suckle their young or again become pregnant. The periovarial membrane is removed. Corpora of pregnancy in each case are blue, apparently somewhat deeper in the older; all subsequently formed corpora are reddish. × ca. 14.

Fig. 59. Portion of a young corpus with the remains of the egg at the center. Although macrophages are loaded with vital dye, the young lutein cells have not stored any of it; given Afridol Blue on the 9th to 12th days after parturition and killed on the 13th. Series 187. Rat 964. × 813. The section was counterstained with alum cochineal.

Fig. 60. Another corpus from the same ovary as shown in figure 59, showing abundant deposit of vital dye in lutein cells, illustrating the fact that older lutein cells have taken up the dye at the same time that younger ones have failed to store it. × 813.

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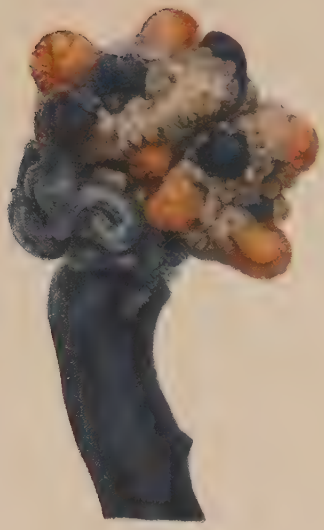

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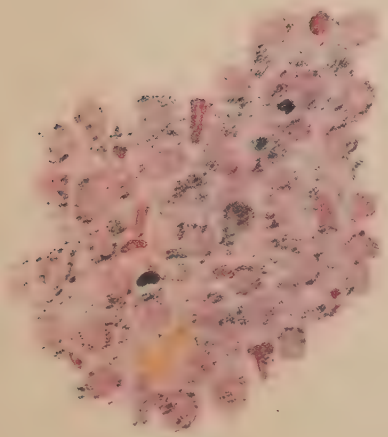

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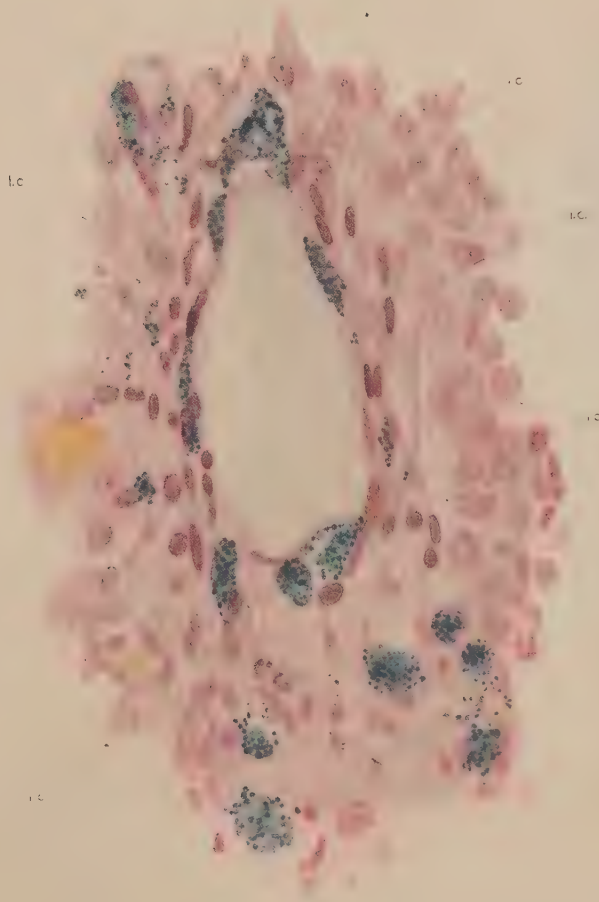

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PLATE VII

Fig. 47. Lipoid granules in lutein cells of a young corpus still containing a large cavity (see fig. 51, pl. VIII) and with the eggs still in the distal fold of the oviduct. Rat 3514. × 1016.

Fig. 48. Lipoid granules in corpus of ovulation in early interval. Lipoid granules small and uniform and moderate in amount (see table 13). Given vital dye from the 9th to 13th day of lactation. Young weaned on 22d day. Stage Three was detected 3 days after weaning. One day later 3 silk loops were placed in the left uterine horn—3 days afterward another Stage Three was detected; animal killed 2 days later within the first hours of the dioestrum. The lutein cells are from the corpus of the second ovulation after weaning. Rat 3533. (See fig. 52, pl. X.) × 1016.

Fig. 49. Lipoid granules in corpus of ovulation at beginning of second cycle, i.e., when corpus is about 4 days old. A new corpus (second ovulation) has just been formed in the same ovary (see fig. 52, pl. X). The granules are larger and more abundant. Rat 3533. × 1016.

Fig. 68. Lipoid granules in lutein cells of corpus of pregnancy on 14th day, and while in full functional vigor. Granules nearly uniform in size and moderate in amount. Note similarity to figure 48, except in greater size of cells. Rat 3473. × 1016.

Figs. 69 and 70. Lipoid granules in outer and inner halves of corpus of pregnancy of rat killed within 12 hours after parturition. The inequality in size appears earlier and is greater in the deeper portions of corpora than in the more superficial parts. The figures show that the size of the granules is suddenly greater and the quantity enormously increased at the end of the period of functional activity. Compare with figure 49, series 428, Rat 1746. × 1016.

Fig. 71. Lipoid granules in a corpus of pregnancy of rat killed 8 days after parturition, i.e., in 2d cycle post-partum. The amount of lipoid is now greatly diminished and the size is very small and still non-uniform. Period of regression. Series 452, Rat 1900. × 1016.

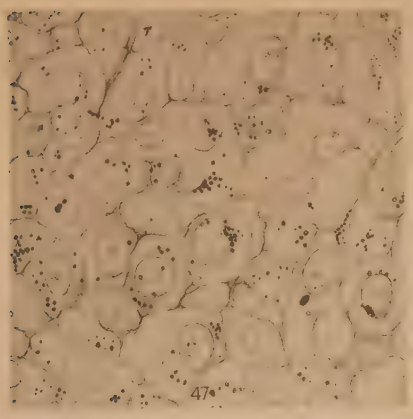
Fig. 79. Lipoid granules of corpus of lactation on 16th day, functionally active. Granules very small (smaller than in any other kind of functional corpus) and uniform, and moderate in amount. Compare with figures 48 and 68, plate VII. Series 374, Rat 1674. × 1016.

Fig. 80. Lipoid granules of corpus of lactation 4 days after weaning. The size and amount has greatly increased after functional activity has ceased. Compare with figures 49, 69, and 70, plate VII. Given vital dye during pregnancy. Allowed to suckle litter of 7 for 6 days, when young removed. Killed 4 days later. Large ripe follicles in ovary and absence of eggs in the oviduct indicate that the first ovulation had not occurred. Rat 3625. × 1016.

Fig. 88. Lipoid in corpus of pseudo-pregnancy 15 days after stimulation, at the first Stage Three, i.e., end of functional activity. Increase in content, size, and inequality of lipoid. Compare with figures 49, 69, 70, and 80, plate VII (see fig. 50, p. 61). Rat 3514. × 1016.

Fig. 81. Lipoid in a corpus of lactation of rat killed 10 days after weaning at the beginning of the second cycle. (See fig. 54, pl. X.) The lipoid granules have now reached the phase in which they are slight in quantity, small and unequal, conditons characteristic of other corpora a cycle after cessation of functioning. (See fig. 71, pl. VII.) Rat 4668. × 1016.

Fig. 87. Lipoid granules in corpus of copulation or pseudo-pregnancy on the 10th day and while still in functional vigor, showing considerable uniformity in size and moderate amount as in other corpora. Compare with figures 48, 68, and 79, plate VII. Glass rod inserted in cervical canal in Stage Two. Four days later 3 silk loops placed through uterine lumen. Killed 6 days afterwards without the incidence of an oestrus. The vaginal smear had been examined daily throughout the period of experimentation. Large placentomata had been produced by the uterine irritation. Rat 3639. × 1016.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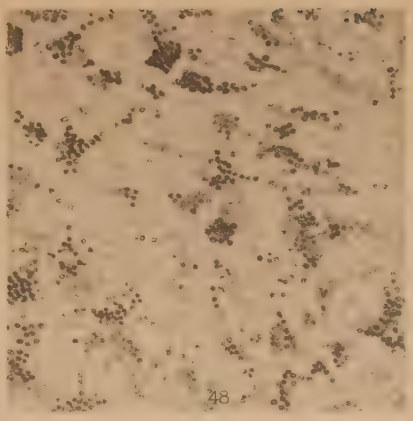
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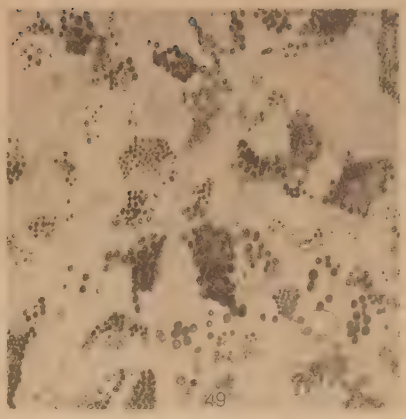
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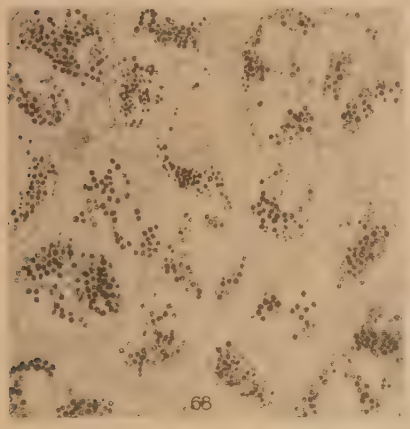
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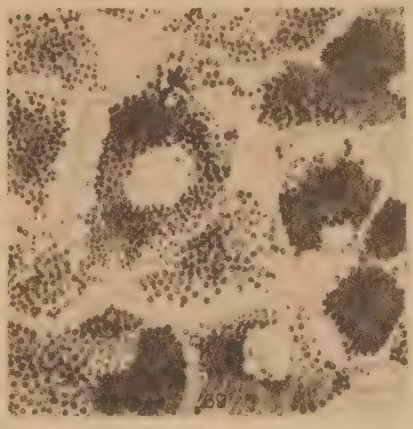
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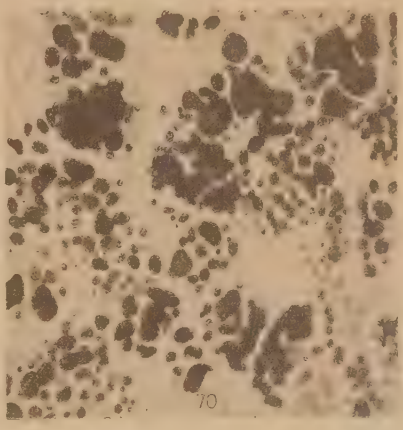
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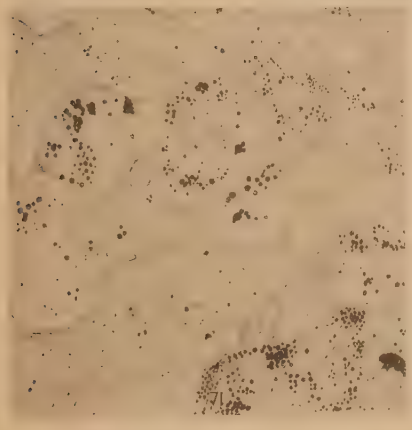
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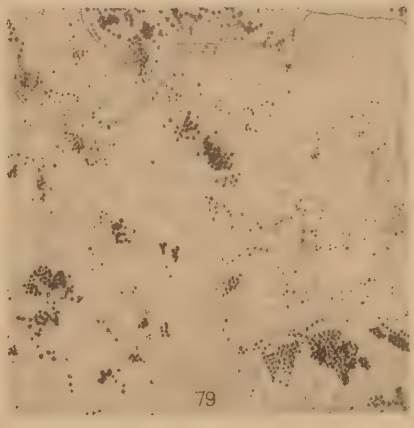
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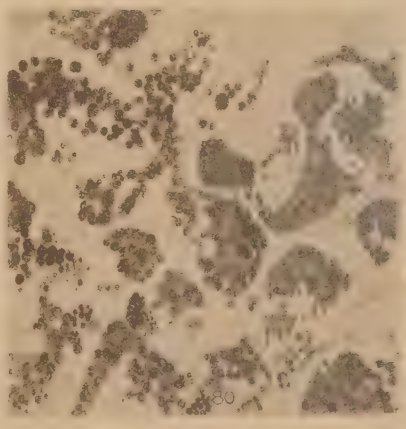
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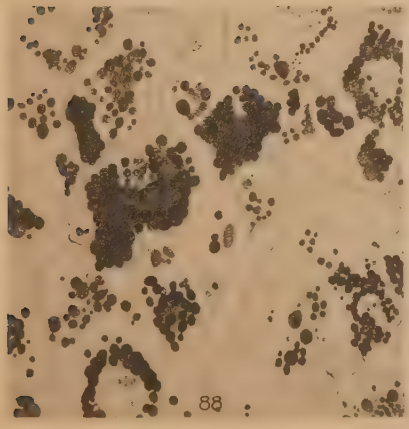
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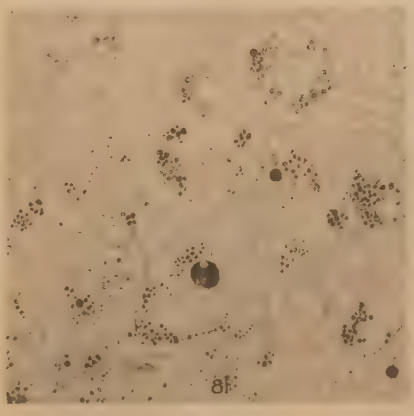
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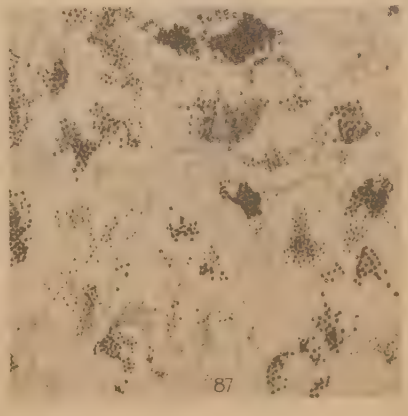
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PLATE VIII

Figs. 16 to 19. Low power photographs of cross-sections of uteri at different times during Stage One to show the increase in size as the result of the accumulation of fluid. Rats 2856, 2797, 2810, 5050. × 9.

Fig. 25. Low-power photograph of cross-section of uterus 20 minutes after copulation to show the maximum distention of uterus with fluid. Diameter, 5 mm. Rat 2454. × 9.

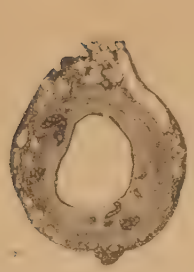
Fig. 29. Photograph of cross-section of uterus in Stage Three. The vaginal smear was observed twice daily for 11 days. The animal was killed in the cheesy stage before the appearance of leucocytes. Rat 3093. × 9.

Fig. 35. Photograph of cross-section of uterus in Stage Four. Rat 2441. × 9.

Fig. 83. Photograph of section of ovary of rat killed on 9th day of lactation. Corpora of pregnancy and of lactation. Series 366, Rat 1368. × 23.

Fig. 72. Photograph of section of ovary of rat killed 3 days after parturition, to show contrast between corpora of pregnancy loaded with lipoid and young corpora of ovulation. Series 367, Rat 1883. × 23.

Fig. 51. Photograph of section through ovary of Rat 3514, AB, figure 50, page 61, to show the appearance at low magnification of the lipoids in corpora of lactation 19 days after weaning; corpora of copulation 15 days old in the beginning of the next cycle when the amount of lipoid is greatly increased (see fig. 87, pl. VII), and very young corpora of ovulation still containing large cavities (fig. 47, pl. VII). × 23.



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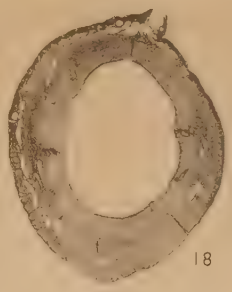
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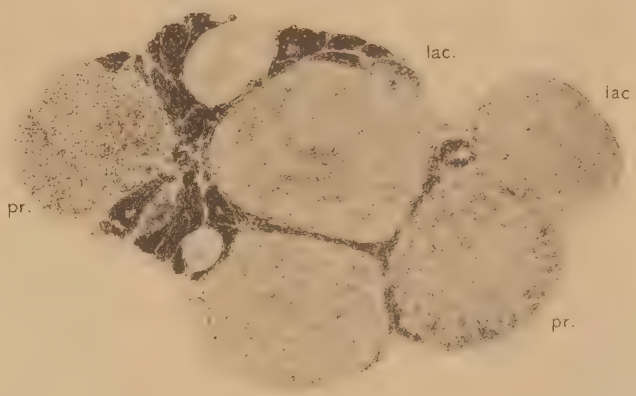
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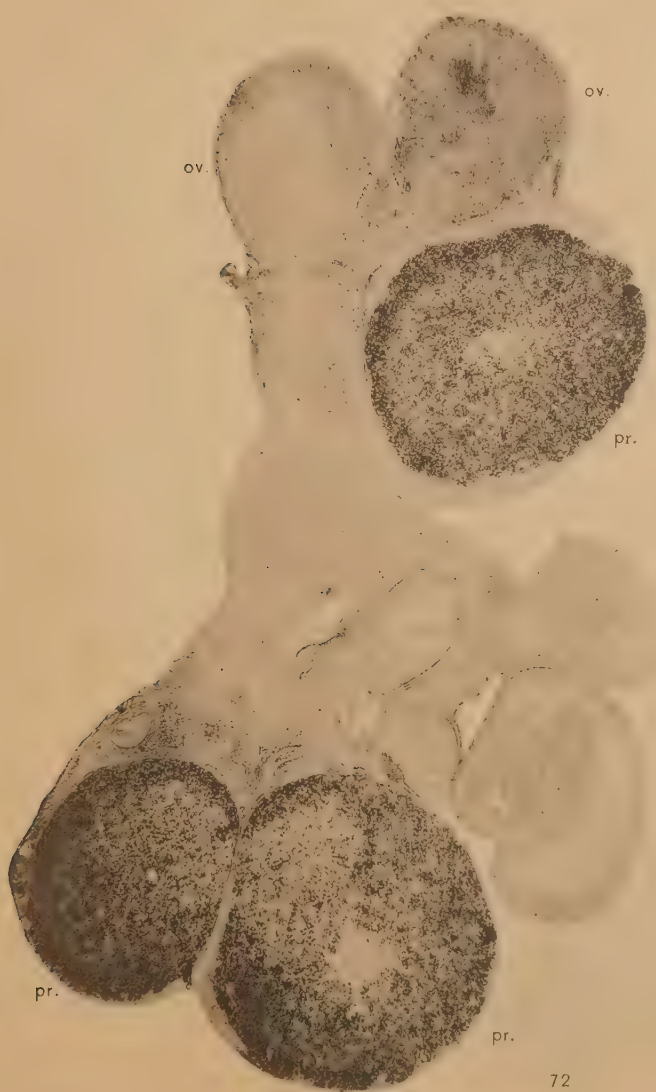
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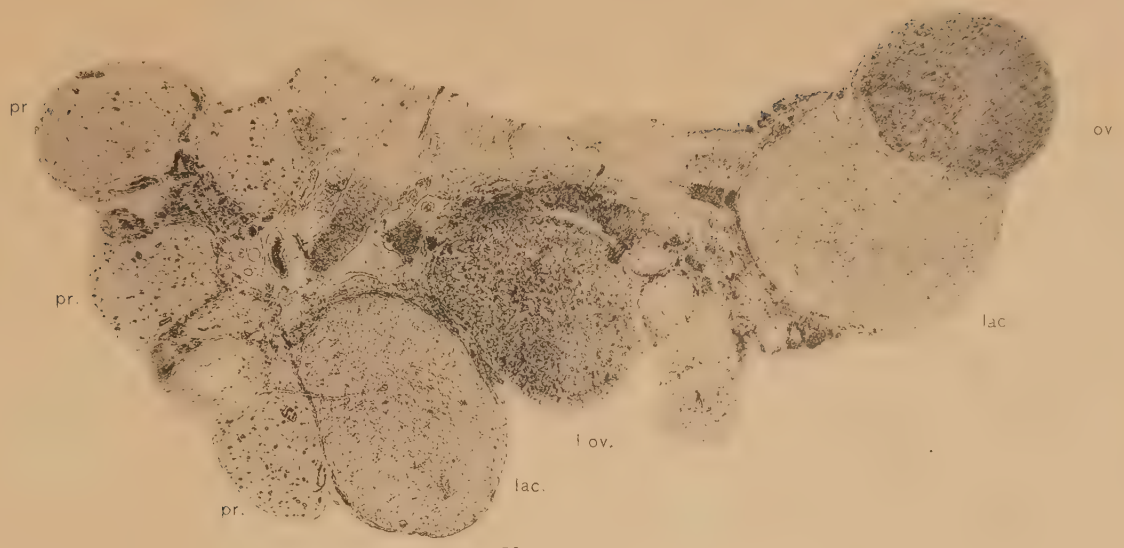
PLATE IX

Fig. 53. Photograph through section of left ovary to show old corpora of pregnancy, corpora of lactation 9 days after weaning, and corpus of first ovulation 5 days old, still in active functioning. Given vital dye during lactation. Young weaned; vaginal smear examined daily; first oestrus 4 days after weaning; left ovary removed 5 days later; second oestrus 3 days after operation; animal killed at third oestrus 5 days later in Stages One-Two. Rat 4675. × 23.

Fig. 89. Photograph of section of ovary of Rat 3514 (fig. 50, C–D, p. 61) to show corpora of copulation (stimulation), corpora of lactation, and old corpus of pregnancy. × 23.

Fig. 96. Photograph of section through an ovary transplanted to the rectus muscle, showing surrounding muscle fibers, follicles with eggs, corpora lutea, and interstitial tissue. The animal was killed at the time of ovulation. Rat 3902. (See table 27.) × 23.



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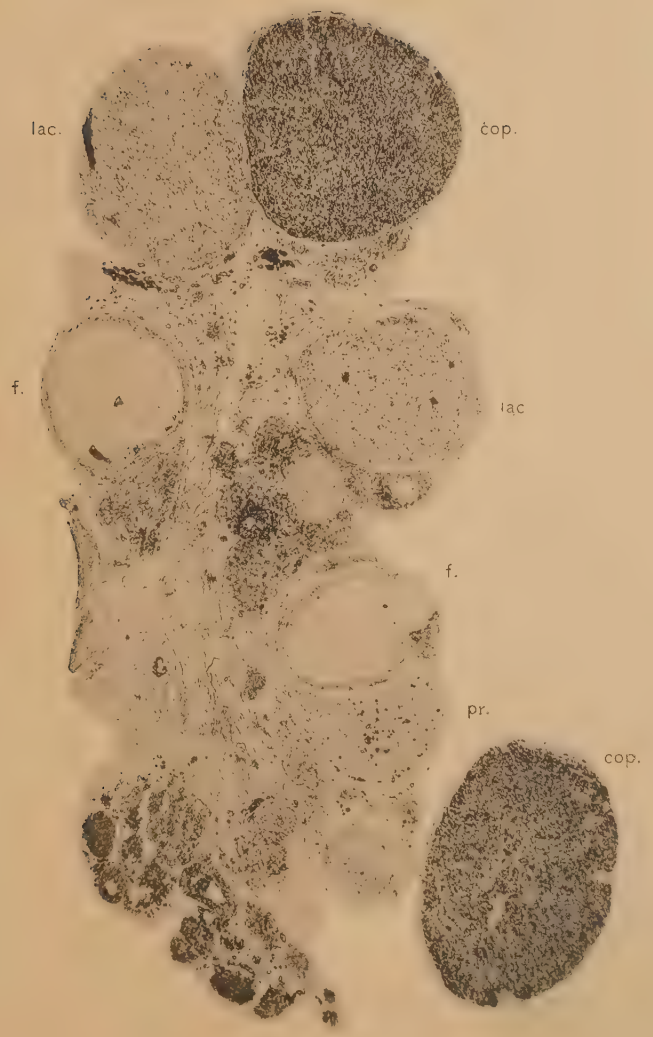

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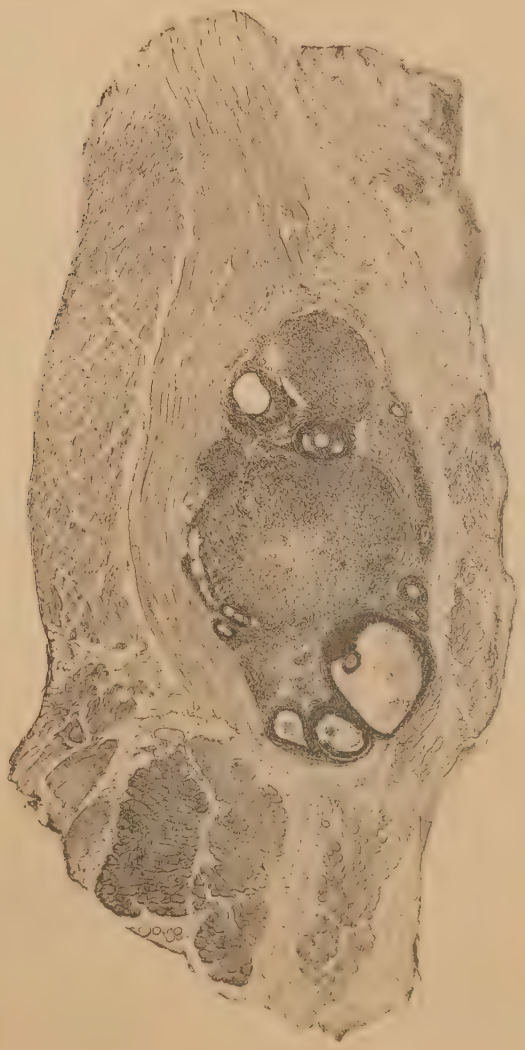

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PLATE X

Fig. 73. Photograph of section of ovary of rat killed 8 days after parturition. Contains four regressing corpora of pregnancy (fig. 71, pl. VII), one corpus of first ovulation, and three corpora of second ovulation. Series 452, Rat 1900. × 23.

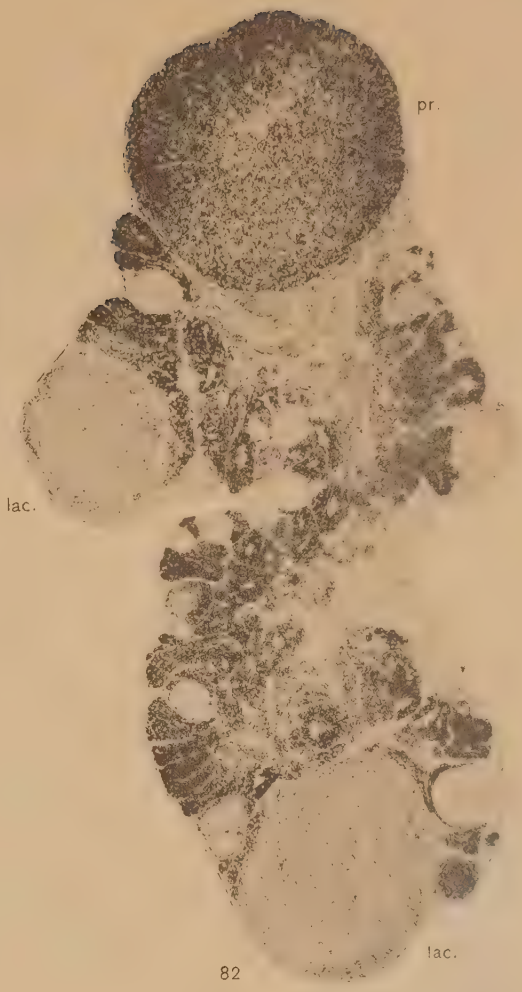
Fig. 82. Photograph of section of ovary of rat killed on the 4th day of lactation, showing corpora of pregnancy and lactation. Series 401, Rat 1737. × 23.

Fig. 54. Photograph of section of ovary showing corpora of pregnancy 31 days after parturition, of lactation 10 days after weaning, and of ovulation at beginning of second cycle (i.e., about 5 days old), but before the next ovulation. Given vital dye during lactation. Weaned, and oestrus 5 days after weaning. Second oestrus 5 days later when killed in Stages One-Two. Rat 4668. × 23.

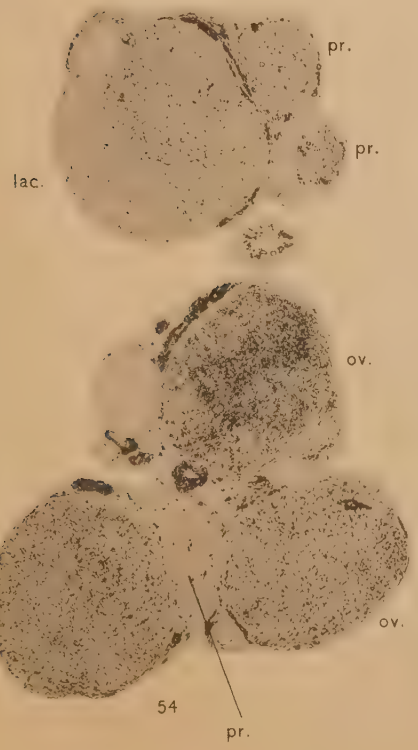
Fig. 52. Photograph of section of ovary to show corpora of lactation 9 days after weaning, of ovulation in early interval (corpora of second ovulation containing lipoid granules as shown in fig. 48, pl. VII), and of ovulation in second cycle (corpora of first ovulation with lipoids shown in fig. 49, pl. VII). The difference in the amount of lipoid in the different corpora is evident. Rat 3533. (For history see fig. 48.) × 23.

2 ov.
pr.
2 ov.
pr.
1 ov.
pr.
pr.
2 ov.

73


pr.
lac.
lac.

82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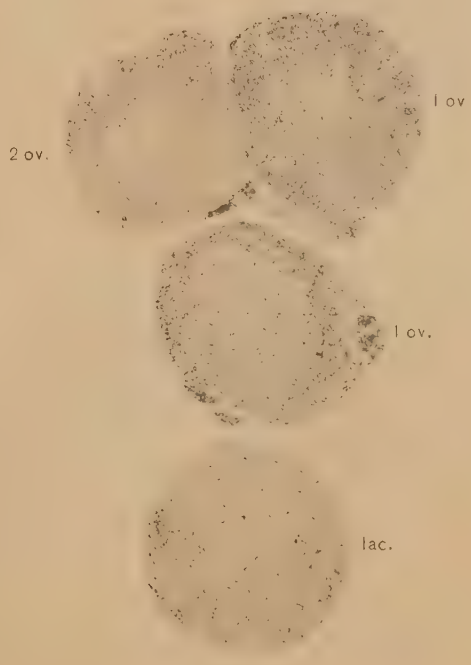
54


1 ov
2 ov.
1 ov.
lac.

52

PLATE XI

Fig. 43. Drawing of egg and surrounding granulosa cells of follicle shown in figure 42. The polar cell which lies on top of the egg is shown at one side. Note the conditions indicated under figure 42. One inpushing of capillaries lies under the discus proligerous. Rat 3784. × 220.

Fig. 85. Photograph of a portion of the margin of a plug in contact with the cornified surface of the vaginal mucosa showing the great similarity between the two substances and the adhering of one to the other. × 215.

Fig. 44. Photograph of young corpus luteum which still contains a large cavity. The periovarial membrane is in contact with the surface of the ovary. The oviduct contains eggs. The vaginal smear was observed at 3 hour intervals for 27 hours. The animal was killed 30 hours after the beginning of Stage Two. Rat 3784. Table 2. × 50.

Fig. 42. Photograph of a follicle containing an egg which has just abstricted the first polar body and is in the process of forming the second maturation spindle. Although the follicle wall is not thin at any place, "ripeness" is further indicated by the loosening of the discus cells from one another and from the rest of the granulosa, and by the inpushings of blood vessels at several places. Rat 3784. See table 2. × 50.

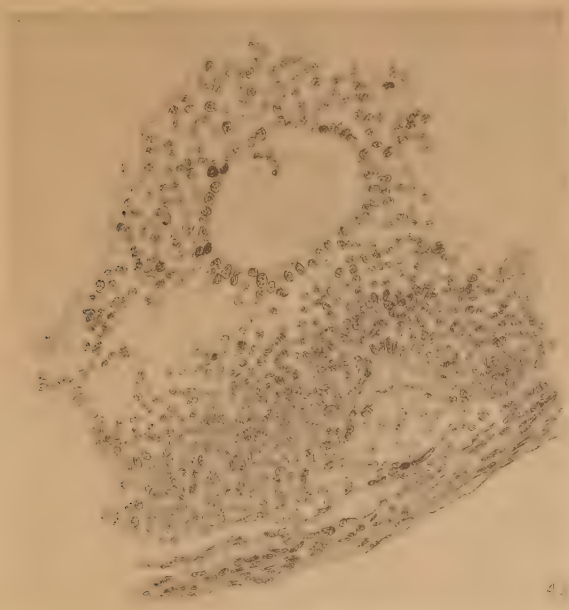
Fig. 95. Decidual cells from a normal pregnancy of 9 days. Rat 2362. (See figure 93.) × 610.

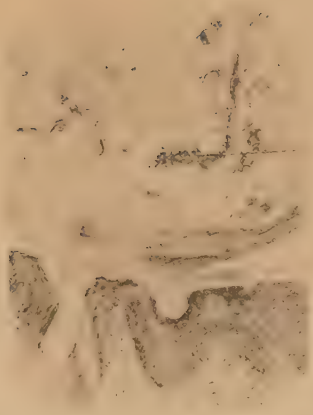
Fig. 93. Photograph of section through a normal pregnant uterus and blastodermic vesicle of rat killed 9 days after copulation. Rat 2362. × 12.

Fig. 92. Photograph of section through placentoma produced by inserting threads into uterus 4 days after stimulation; animal killed 6 days later. The small cross (×) marks the position of thread. Note similarity to normal pregnancy in figure 93. Rat 3639. × 12.

Fig. 94. Decidual cells from area indicated by small circle in figure 92. × 610.

Fig. 45. A degenerating egg at the center of a corpus luteum. The cytoplasm has undergone fragmentation as do eggs in the oviduct, and contains several small nucleus-like bodies. The zona pellucida is represented by a thin band surrounding the egg. Fixed in Benda's fluid. Given vital dye during pregnancy. Killed 4 days after parturition before the occurrence of the second post-partum ovulation; the corpus luteum represented is one of the post-partum ovulation set. Series 453, Rat 1952. × 610.





Spermatozoa.

Cornified layer adhering to plug.

Cornified layer.

Stratified mucosa.

85

94

93

97

45

TABLE 35

Conditions found in the reproductive organs of animals ki

Desig-nation of animal	Stage	Hours since beginning of preceding stage	Behavior	Smear			Thickness	No. of cell layers	Vagina		
				Leucos.	Epith.	Cornif.			Leucocytes—In section	"Superficial epithelium"	Stratum
3498	Middle int.	ca. 96 hrs.		+	+		42 micra	4-5	Many in mucosa and stroma	None	Absent
2263	End of int.			+	few		51-54 mic.	7-8	Many in mucosa and stroma	Present	Absent
3703	"early 1"	ca. 1 hr.	Not in heat		+		84-92 mic.	8-9	Some in mucosa and stroma	Beginning	Absent
2856	1				+		92 micra	9-11	Few in surface	Adhering in places	Thin; well for
2290	Late 1				+	+	84 micra	9-12	Occasional in cornified layer	Adhering in places	Well formed
3712	1	ca. 1 hr.	Not in heat		+		92 micra	10-12	Very infrequent	Mostly adherent	Adherent
2810	1				+	few	93 micra	8-11	None	Practically all loose	Adherent
5050	1				+	few	115 micra	9-12	None	Entirely detached	Adherent; spli
2797	2						73-84 mic.	8-12	None	None	Adherent
3670	2	ca. 33 hrs.	Beginning of œstrus			+	115 micra	10-12	None	None	Thick, adhere
3612	2	ca. 33 hrs.	Early œstrus			+	92 micra	9-11	None	None	Adherent, gre
3590	2	ca. 24 hrs.	Early œstrus		few	+	107 micra	8-10	None	None	Adherent, not
3683	2	ca. 30 hrs.	Mid-œstrus			+	100 micra	8-10	None	None	Adherent, not
2454	2		Plug			+	100 micra	8-11	None	None	Adherent, par
3693	2	ca. 87 hrs.	Plug; mid-œstrus			+	107 micra	9-11	None	None	Adherent
3715	Middle 2	ca. 75 hrs.	Mid-œstrus			+	84 micra	9-10	None	None	Adherent, thi
3574	Late 2	ca. 30 hrs.	End of œstrus			+	84 micra	7-9	None	None	Adherent, thi
3675	2	82 hrs.	End of œstrus			+	75 micra	7-9	None	None	Completely st
3093	3	24-30 hrs.				cheesy +	92 micra	9	None	None	Partly adhere
3689	3	87 hrs.				cheesy	53 micra	5-7	None	None	Completely st
3685	3	60 hrs.				cheesy	46 micra	6-8	None	None	Completely st
3678	4	65 hrs.			+	+	88 micra	9	None	None	Completely st
2441	early 4			+		+	76 micra	5-8	Very few in deep layers	None	Partly adhere
2879	late 4			+		+	69 micra	6-8	Very few	None	Some still adh nearly al
3745	early 4	67 hrs.		+		+	76 micra	7-9	Few deep	None	Partly strippe
3718	early 4	49 hrs.		+		+	61 micra	5-7	Many	None	Absent
3647	early 4	56 hrs.		+	+	+	65 micra	5-7	Some	None	Absent
3716	end 4	63 hrs.		+	+	–	57 micra	4-6	Some	None	Absent
3719	late 4	64 hrs.		+	+	–	50 micra	6-7	Some	None	Absent
3673	end 4	120 hrs.		+	+		88 micra	8-9	None	None	Absent
3649	late 4	78 hrs.		+	+		65 micra	6-8	Some	None	Absent
3671	late 4	61 hrs.		+	+		46 micra	6-7	Many	None	Absent
3711	end 4	63 hrs.		+	+		42 micra	5-6	Many	None	Absent
3672	late 4	90 hrs.		+	+		50 micra	7	Very many	None	Absent
2862	very late 4			+	+	few	65 micra	6-8	Very many	None	Absent
3506	early inter.	ca. 48 hrs.		+	+		53 micra	5-8	Many	None	Absent
2445	dicestrum			+	+		53 micra	5-7	Very many	None	Absent
2905	later di-œstrum						69 micra	6-8	Very many	None	Absent

led at different times during the œstrous cycle.

		Mitoses counted in one section	Uterus		Oviduct		Ovary	Rat No.
ornium	Stratum granulosum		Diameter on slide	Character of epithelium	Degree of distention	Position of eggs	Follicles	
	Absent	3	1.7 mm.	Healthy	Normal	Have reached proximal part of oviduct	Medium .45 mm.	3498
	Absent	23	3.4 mm.	Healthy	Normal	None	Medium .50 mm.	2263
	Occasional granules	17-19	2.3 mm. no fluid	Healthy	Normal	None		3703
ned	Present	5	2.5 mm. fluid	Healthy	Normal	None	Medium .65 mm.	2856
	Distinct	12	3.3 mm. fluid	Congestion. Sub-epithelial hemorrhage	Normal	None	Medium .65 mm.	2290
	Distinct	10	2.0 mm.	Healthy	Normal	None	Medium .65 mm.	3712
	Distinct	3	3.4 mm. fluid	Healthy	Normal	None	Medium .65 mm.	2810
tting	Distinct	7	3.7 mm. fluid	Healthy	Normal	None	Large .75 mm.	5050
	Distinct	7	2.8 mm. flaccid	Healthy	Normal	None	Medium .70 mm.	2797
it, splitting	Distinct	9	2.5 mm.	Healthy	Normal	None		3670
itly split	Distinct	8	3.8 mm.		Normal	None		3612
much split	Distinct	3	3.4 mm.	Healthy	Normal	None	Large .75 mm.	3590
much split	Distinct	4	Not cut					3683
tly split	Distinct	4	5.0 mm.	Healthy	Normal	None	Large .75 mm.	2454
	Distinct	1	2.7 mm. fluid	Uterus full of blood. Healthy	Not cut			3693
k, splitting	Distinct	4	1.7 mm. no fluid	Vacuolar degeneration begun	Normal	None	1st polar spindle .90 mm.	3715
k, splitting	Distinct	4	1.8 mm.	Vacuolar degeneration		Distal fold	Small .40 mm.	3574
ripped	Remnants	6	2.3 mm.	Vacuolar epithelium		Distal fold	Small .50 mm.	3675
nt; split	Greatly reduced	1	2.9 mm.	Subepithelial haemorrhage. H'lthy	Normal	None	Medium .70 mm.	3093
ripped	None	2	1.7 mm.	Healthy	Normal	Entering intermediate portion		3689
ripped	None	6	1.4 mm.	Vacuolar degeneration extensive	Not cut			3685
ripped	None	7	2.3 mm.	Fairly healthy	Normal	Far along in oviduct	Enormous atretic follicles containing eggs	3678
nt; splitting	None	2-4	2.2 mm.	Healthy	Distended	Distal fold		2441
rent although detached d	None	1	1.7 mm.	Vacuolar degeneration	Distended	Proximal portion of distal fold	Small	2879
	Remnants	7-9	2.0	Some vacuolar degeneration	Distended	Distal fold		3745
	None	2-3	2.5 mm.		Distended	Distal fold		3718
	None	5	2.5	Vacuolar degeneration extensive	Distended	Distal fold		3647
	None	10				Not cut		3716
	None	14	2.7 mm.	Some vacuolar degeneration	Distended	Distal fold		3719
	Occasional vestige	17	2.0 mm.	Healthy	Normal	None	Medium .60 mm.	3673
	None	16	2.0 mm.	Some vacuolar degeneration	Normal	About middle		3649
	None	12-15	2.7 mm.	Beginning vacuolar degeneration	Distended	Distal fold		3671
	None	3	1.6 mm.	Some vacuolar degeneration				3711
	None	4	2.0 mm.		Normal	None	Large .75 mm.	3672
	None	5-10	1.7 mm.	Vacuolar degeneration still extensive	Distended	Proximal position of distal fold	Small	2862
	None	5	2.3 mm.	Healthy	Normal	About middle of oviduct		3506
	None	7-10	2.5 mm.	Healthy	Somewhat distended	Leaving distal fold	Small	2445
	None	7	1.7 mm.	Healthy	Normal	Well along in oviduct		2905

APPENDIX

TABLES 35, 36 AND 37

TABLE 36.

Table showing vital dyes employed to stain corpora lutea.

Chemical classification of vital dyes employed	Exact chemical constitution of dye	Name or designation of dye	General vital staining properties	Macroscopic vital stain of corpora as seen in fresh tissue	Presence of vital dye granules in the fresh lutein cells	Fixation and preservation of vital dye granules in sections
Symmetrical Benzidine dyes made by combining a para-diamine base with amido-naphthol disulfonic acids	(1) Tolidine + 2 molecules 1.8 amido-naphthol 2.4 disulfonic acid	T.1.8.2.4.	Excellent bright blue general stain	Probably satisfactory		Unsatisfactory
	(2) Tolidine + 2 molecules 1.8 amido-naphthol 3.5 disulfonic acid	T.1.8.3.5.	Excellent blue general stain	Probably satisfactory		Unsatisfactory
	(3) Tolidine + 2 molecules 1.8 amido-naphthol 3.6 disulfonic acid	Trypan blue	Excellent general stain	Good general stain of corpora	Weakly stained granules in lutein cells of normal ovulation. In one instance corpora of lactation sharp blue granules	Unsatisfactory
	(4) Tolidine + 2 molecules 1.8 amido-naphthol 4.6 disulfonic acid	T.1.8.4.6	Light violet general stain	Corpora a distinct grayish violet. Other samples of dye stained them a much deeper color	Lutein cells contain many pale granules of dye, fairly sharp	Unsatisfactory; the dye granules diffuse so as to look like a vital plasma stain in sections
	(5) Tolidine + 2 molecules 2.8 amido-naphthol 3.6 disulfonic acid	224	Pale general stain	Satisfactory	Lutein cells containing *very* pale granules	Unsatisfactory
	(6) Tolidine disulfonic acid + 2 molecules 1.8 amido-naphthol 3.6 disulfonic	LT 297 acid	Pale general stain	Pale	Unsatisfactory	Unsatisfactory
	(7) Benzidine-meta-disulfonic acid + 2 molecules H acid	225	Faint general stain	Satisfactory	Lutein cells contain very faint, fine granules, presumably pinkish	Unsatisfactory; even the pale violet granules in marcophages of atresia are not well fixed but diffuse
	(8) Benzidine-meta-disulfonic acid + 2 molecules 1.8 amido-naphthol 4.6 disulfonic acid	226	Deep general stain	Pale	No granules	Unsatisfactory; even the macrophage granules of atresia are not well fixed
	(9) Dianisidine + 2 molecules 1.8 amido-naphthol 2.4 disulfonic acid	Chicago blue 6B	Excellent blue general stain	Splendid vital stain of corpora	Fair quantity of robins-egg blue, round granules in all corpora cells; germinal epithelium with deeper brilliant blue granules	Unsatisfactory

	(10) Dianisidine + 2 molecules 1.8 amido-naphthol 3.6 disulfonic acid	Benzamine rein blau FF	Excellent pure blue general stain	Corpora lutea deep pure blue	Lutein cells contain fairly numerous pale blue granules, some are bright blue	Unsatisfactory
	(11) Dichlor Benzidine + 2 molecules of 1.8 amido-naphthol 3.6 disulfonic acid	**Afridolblau**	**Pale blue general vital stain**	**Deep purplish blue**	**Lutein cells with many small sharp, deep blue granules**	**Well fixed and preserved in sections in all methods employed. (Dye is toxic to animal.)**
Symmetrical Benzidine dyes made by combining a paradiamine base with 2 molecules of an amido-naphthol monosulfonic acid	(12) Benzidine + 2 molecules 2.8 amido-naphthol 6 monosulfonic acid	Naphthamine Black BVE	Does not give a general vital stain	Unsatisfactory	Unsatisfactory	Unsatisfactory
	(13) Benzidine-monosulfonic acid + 2 molecules 2.8 amido-naphthol 6 monosulfonic acid	150	Weak general stain	Unsatisfactory	Unsatisfactory	Unsatisfactory
	(14) Benzidine-monosulfonic acid + 2 molecules 2.5 amido-naphthol 7 monosulfonic acid	151	Very weak general stain of animal	Unsatisfactory	Extremely pale lavender granules in lutein cells—too weak to be useful	Unsatisfactory. Atresia shown only by pale rose granules in macrophages
	(15) Benzidine-meta-disulfonic acid + 2 molecules 2.8 amido - naphthol 6 monosulfonic acid	227	Excellent deep general stain of animal	Satisfactory	Deep red, small, angular dye deposits in lutein cells	Unsatisfactory, but follicular atresia splendidly marked by vital stain of macrophages
	(16) Benzidine-meta-disulfonic acid + 2 molecules 2.5 amido - naphthol 7 monosulfonic acid	228	Deep general stain	Deep color of corpora	Some pink granules in lutein cells; in fact, under low power cells appear full of granules	Unsatisfactory
	(17) Tolidine + 2 molecules 1.8 amido - naphthol 4 monosulfonic acid	Chicago blue R	Not a general vital stain	Unsatisfactory	Unsatisfactory	Unsatisfactory
	(18) Tolidine + 2 molecules 2.8 amido - naphthol 6 monosulfonic acid	Naphthamine Black TBVE	Not a general vital stain	Unsatisfactory	Unsatisfactory	Unsatisfactory
Symmetrical Benzidine dyes made by combining a paradiamine base with 2 molecules of naphthylamine disulfonic acids	(19) Dichlor Benzidine + 2 molecules Beta-naphthylamine 3. 6disulfonic acid	Dianolbrilliantred X	Light general stain	Pale pink corpora	Unsatisfactory	Unsatisfactory; even granules in macrophages are very pale pink and diffuse

TABLE 36—Continued.

Table showing vital dyes employed to stain corpora lutea.

Chemical classification of vital dyes employed	Exact chemical constitution of dye	Name or designation of dye	General vital staining properties	Macroscopic vital stain of corpora as seen in fresh tissue	Presence of vital dye granules in the fresh lutein cells	Fixation and preservation of vital dye granules in sections
	(20) Dichlor Benzidine + 2 molecules Beta-naphthylamine 3.6 disulfonic acid	Dianil brilliantred R	Light general stain	Pale pink	No granules in lutein cells	No granules
	(21) Benzidine-monosulfonic acid + 2 molecules Beta-naphthylamine 3.6 disulfonic acid	Trypan red	Pale pink general stain	Pale pink stain of corpora	Very faint pink, scanty granules in lutein cells	Unsatisfactory
	(22) Benzidine + 2 molecules Beta-naphthylamine 5.7 disulfonic acid	4 (Hoechst)	No general color	No stain of corpora	No granules	No granules. Atresia only shown by very pale lavender granules
	(23) Tolidine + 2 molecules Alpha-naphthylamine 4.8 disulfonic acid	T.1.4.8	Deep red vital stain	Pale red corpora	No sharp granules	Unsatisfactory
	(24) Tolidine + 2 molecules Beta-naphthylamine 3.6 disulfonic acid	T.2.3.6	Light red general stain	Unsatisfactory	Unsatisfactory	Unsatisfactory
	(25) Tolidine + 2 molecules Beta-naphthylamine 5.7 dusulfonic acid	T.2.5.7	Pale general stain	Deep color, apparently diffuse. ANOTHER SAMPLE: entirely negative	Granules pale yellow, even in macrophages	Unsatisfactory
	(26) Tolidine-disulfonic acid + 2 molecules Beta-naphthylamine 3.6 disulfonic acid	Tolidine-disulfonic acid plus Beta-naphthylamine 3.6	Deep orange general stain	Presumably diffuse orange stain in corpora	Unsatisfactory	Unsatisfactory
Symmetrical Benzidine dyes made by combining a para-diamine base with 2 molecules of a naphthylamine monosulfonic acid	(27) Benzidine-ortho-disulfonic acid + 2 molecules Beta-naphthylamine 7 monosulfonic acid	230	Animal deep crimson	Diffuse stain in corpora	No granules in lutein cells	No granules. Atresia deep pink, but diffuse
	(28) Benzidine-meta-disulfonic acid + 2 molecules Beta-naphthylamine 7 monosulfonic acid	229	Animal palely stained	Viscera not deeply stained	Lutein cells devoid of dye deposits	Entirely negative
	(29) Tolidine + 2 molecules Alpha-naphthylamine 6 monosulfonic acid	5 (440)	Very pale	Pale general stain	Lutein cells contain pale yellowish and brown granules	Unsatisfactory

	(30) Tolidine + 2 molecules Beta-naphthylamine 7 monosulfonic acid	10 (445)	Very pale general stain of animal	Unsatisfactory	No granules	No granules; even atresia not bright
	(31) Tolidine + 2 molecules Beta-naphthylamine 8 monosulfonic acid	11 (9561)		Corpora lutea pale pink, no more intense than remainder of viscera	Unsatisfactory	Unsatisfactory; even atresia very weak
Symmetrical Benzidine dyes made by combining a paradiamine base with 2 molecules of a naphthol disulfonic acid	(32) Tolidine + 2 molecules Alpha-naphthol 3.6 disulfonic acid	W.T.M. III	Very faint lavender	Unsatisfactory	No granules	Negative; even atresia pale pink, tending to be diffuse
Symmetrical Benzidine dyes made by combining a paradiamine base with 2 molecules of a naphthol monosulfonic acid	(33) Benzidine + 2 molecules Beta-napthhol 8 monosulfonic acid	Neubordeaux L Tech. Rein.	Light general stain	Unsatisfactory	No granules	No granules; even atresia with only pale pink granules
Symmetrical Benzidine dyes made by combining 1 molecule of a paradiamine base with 2 molecules of a naphthol trisulfonic acid	(34) Benzidine + 2 molecules Alpha-naphthol 3.6.8 trisulfonic acid	Trisulfonazoblau	Light general stain	Ovary light blue	Corpora with no granules	Negative
Asymmetrical Benzidine dyes made by combining a paradiamine base with 1 molecule of an amidonaphthol disulfonic acid acid and a naphthylamine, naphthol, or amidonaphthol	(35) Benzidine + 1.8 amidonaphthol 3.6 disulfonic acid and 2.8 Ethylamidonaphthol 6 monosulfonic	316 (Diphenylblauschwarz)	Purple general stain	Unsatisfactory	Unsatisfactory	Unsatisfactory
	(36) Benzidine + 1.8 amidonaphthol 3.6 disulfonic acid and Alpha-naphthylamine	193 Azo mauve R.	Weak	Unsatisfactory	Unsatisfactory	Unsatisfactory
	(37) Benzidine + 1.8 amidonaphthol 4.6 disulfonic acid and 2.8 amidonaphthol 6 monosulfonic acid	Naphtamine black RE	Light dusky blue	Probably satisfactory	Unsatisfactory	Unsatisfactory
	(38) Benzidine + 1.8 amidonaphthol 3.6 disulfonic acid and 2.8 amidonaphthol 6 monosulfonic acid	Naphthamine black CE	Light dusky blue	Corpora tissue contains a large amount of dye	Deposits in lutein cells are very pale	Unsatisfactory

TABLE 36—Continued.

Table showing vital dyes employed to stain corpora lutea.

Chemical classification of vital dyes employed	Exact chemical constitution of dye	Name or designation of dye	General vital staining properties	Macroscopic vital stain of corpora as seen in fresh tissue	Presence of vital dye granules in the fresh lutein cells	Fixation and preservation of vital dye granules in sections
	(39) Benzidine + 1.8 amido-naphthol 3.6 disulfonic acid and 2.8 amidonaphthol 6 monosulfonic acid	Diamine black BH	Light dusky blue	Probably satisfactory	Unsatisfactory	Unsatisfactory
	(40) Tolidine + 1.8 amido-naphthol 3.6 disulfonic acid and Alphanaphthol 4 monosulfonic acid	Naphtamine blue BXR	Light dusky blue	Probably satisfactory	Unsatisfactory	Unsatisfactory
	(41) Tolidine + 1.8 amido-naphthol 3.6 disulfonic acid and Beta-naphthylamine	Naphtazurine BN Naphazurine BNOOO	Very pale stain	Unsatisfactory	Unsatisfactory	Unsatisfactory
	(42) Dianisidine + 1.8 amidonaphthol 2.4 disulfonic acid and 1.8 amidonaphthol 4 monosulfonic acid	Chicago blue 4B	Bright blue	Corpora bright blue	Abundant greenish-blue granules in all lutein cells	Unsatisfactory
	(43) Dianisidine + 2amido-do - metatoluylene - diamine 8 naphthol 3.6 disulfonic acid and metatoluylene-diamine	Direct blue black B	Very pale	Pale	Probably some pale granules in corpora	Unsatisfactory
Asymmetrical Benzidine dyes made by combining a paradiamine base with 1 molecule of an amido-naphthol monosulfonic acid and a naphthylamine naphthol, or amidonaphthol	(44) Benzidine + 2.8 amidonaphthol 6 monosulfonic acid and Betanaphthylamine 3.6 disulfonic acid	Dianil granat B	Animal not appreciably stained	Unsatisfactory	Unsatisfactory	Negative; even atresia in sections very poor
	(45) Benzidine + Salicylic acid and 2.8 amidonaphthol 6 monosulfonic acid	Diamine fast Red F recryst.	No general stain	Unsatisfactory	Unsatisfactory	Unsatisfactory
	(46) Tolidine + Alphanaphthol 3.8 disulfonic acid and 1.8 amidonaphthol 4 monosulfonic acid	Columbia blue G	Pale dusky blue stain	Probably satisfactory	Unsatisfactory	Unsatisfactory

Asymmetrical Benzidine dyes made by combining a paradimine base with 1 molecule of naphthylamine disulfonic acid and 1 molecule of naphthylamine monosulfonic acid	(47) Tolidine + Alphanaphthylamine 4 monosulfon-acid and Betanaphthylamine 3. 6 disulfonic acid	Brilliant purpurine R	Deep pink	Light color, presumably diffuse	Unsatisfactory	Unsatisfactory
	(48) Benzidine + Betanaphthylamine 6 monosulfonic acid and Betanaphthylamine 3.6 disulfonic acid	86 (Brilliant Congo G)	Deep pink	Probably satisfactory	Unsatisfactory	Unsatisfactory
	(49) Tolidine + Betanaphthylamine 3.6 disulfonic acid and Betanaphthylamine 6 monosulfonic acid	Brilliant Congo R	Deep pink	Probably satisfactory	Unsatisfactory	Unsatisfactory
Asymmetrical Benzidine dyes made by combining a paradiamine base with 1 molecule of a naphthol monosulfonic acid and 1 molecule of a naphthylamine, naphthol, or amidonaphthol.	(50) Benzidine + Chromotrope acid and Betanaphthol 6 monosulfonic acid	111 (Dianilblau 4R)	Palest bluish violet	Corpus very pale blue	Every cell with a few deep blue, small granules, but germinal epithelium well stained	Granules fixed in sections
	(51) Benzidine + Alphanaphthol 4.8 disulfonic acid and Betanaphthol 8 monosulfonic acid	197 (Heliotrope 2B)	Deep pink	Unsatisfactory	Unsatisfactory	Negative
	(52) Tolidine + Chromotrope acid and Alphanaphthol 4 monosulfonic acid	**112 Dianilblau 2R**	**Pale dusky blue**	**Deep blue**	**Every lutein cell with many small spherical dusky blue granules so that even low power shows a "haze" of fine deposite**	**Excellent preservation by all methods tried. Nontoxic**
	(53) Dianisidine + Alphanaphthol 4 monosulfonic acid and Betanaphthol 3.6 disulfonic acid	117 (Congoblau 2B)	No general stain	Pale violet corpora	No granules	Negative
	(54) Dianisidine + 1.7 Dioxyl-2 Naphtoe 4 sulfonic acid and Alphanaphthol 4 monosulfonic acid	221 (Indazurine GM)	Pale greenish blue	Unsatisfactory	Unsatisfactory	Unsatisfactory
	(55) Tolidine + Alphanaphthol 4 monosulfonic acid and 1.8 amidonaphthol 3.6 disulfonic acid	294 - SHH (Diamine blue BX)	Pale dusky blue	Probably satisfactory	Unsatisfactory	Unsatisfactory

TABLE 36—Continued.

Table showing vital dyes employed to stain corpora lutea.

Chemical classification of vital dyes employed	Exact chemical constitution of dye	Name or designation of dye	General vital staining properties	Macroscopic vital stain of corpora as seen in fresh tissue	Presence of vital dye granules in the fresh lutein cells	Fixation and preservation of vital dye granules in sections
Asymmetrical Benzidine dyes made by combining 1 molecule of a naphthol trisulfonic acid and Beta-naphthol	(56) Benzidine + Beta-naphthol and Alphanaphthol 3.6.8 trisulfonic acid	Trisulfon violet B	Pale violet general stain	Pale violet stain of corpora (corpora somewhat reddish)	Scanty, pale reddish granules in lutein cells	Granules are diffuse in sections
	(57) Tolidine + Betanaphthol and Alphanaphthol 3.6.8 trisulfonic acid	Trisulfonblau R	Pale general stain	Pale stain of corpora	Probably granules, but very pale	Negative
	(58) Dianisidine + Beta-naphthol and Alphanaphthol 3.6.8. trisulfonic acid	Trisulfonblau B	Pale general stain	Pale stain of corpora	Scanty, extremely pale granules	Negative
Asymmetrical Benzidine dyes made by combining a paradiamine base with 1 molecule of a naphthol disulfonic acid and a substituted benzidine or naphthamine	(59) Diethylbenzidine + Phenol and Betanaphthol 6.8 disulfonic acid	Diamine scarlet B		Bright vermillion vital stain of corpus	No granules	Negative
	(60) Dianisidine + 1.7 Dioxy 2 Naphthoe acid 4 sulfonic acid and Beta-naphthol 3.6 disulfonic acid	Indazurine 2B	Animal light greenish blue	Corpora bright blue	Lutein cells contain many small, moderately deep blue, sharp granules	Negative
Stilbene dyes	(61) Para - para - diamido-stilbene + 2 molecules 1.8 amidonaphthol 3.6 disulfonic acid	155	Light blue	Corpora fairly well stained	Granules too faint for use	Negative
	(62) Diamido - stilbene -disulfonic acid + 2 molecules Betanaphthylamine	Hessian purple N		Unsatisfactory	Unsatisfactory	Unsatisfactory
		Hessian purple B		Unsatisfactory	Unsatisfactory	Unsatisfactory
Urea Azo dyes	(63) Diamido-diphenyl-urea-disulfonic acid + 2 molecules 2.8 amidonaphthol 6 monosulfonic acid	Benzo fast pink 2BL	Pale pink general stain	Bright pink corpora	Granules are scanty and deep red	Unsatisfactory
	(64) Para-para-diamido-diphenyl-urea + 2 molecules 1.8 amidonaphthol 3.8 disulfonic acid	101 (Trypan violet)	Pale violet	Pale reddish corpora	Occasional red granules in lutein cells	Unsatisfactory

	(65) Para-para-diamido-diphenyl-urea-meta-meta-disulfonic acid + 2 molecules of a naphthylamine monosulfonic acid	VNR	Excellent vital stain	Pale general stain	Unsatisfactory	Unsatisfactory
	(66) Sulfo-meta-toluylene-diamine - dicarbonyl - dioxy-dinaphthylamine-disulfonic acid + Orthotolidine and Betanaphthylamine	300 Azidinechtscharlach 4 BS (Jaeger)	No general stain	Corpora red to naked eye	Granules too faint for use	Negative but atresia red; fairly good
	(67) Sulfo-meta-toluylene-diamine - dicarbonyl - dioxy-dinaphthylamine-disulfonic acid and 2 molecules Betanaphthylamine	301 (Azidinechtscharlach 7 BS)		Unsatisfactory	Unsatisfactory	Unsatisfactory
Disazo dyes made from Diamines	(68) Diamido - dixlyl - phenyl-methane + 2 molecules Betanaphthol 3.6 disulfonic acid	303	Pale general stain	Pale reddish stain of corpus	Granules pale yellow	Unsatisfactory; even atresia only pale pink
	(69) Diamido - dixlyl - methane + 2 molecules Betanaphthol 3.6 disulfonic acid	304	Diffuse vital stain	Diffuse red stain of corpus	No granules	Negative but atresia bright crimson
Other Azo dyes of known constitution	(70) 1 naphthylamine + 4 monosulfonicacidand1 .8 amidonaphthol 3.6 disulfonic acid and Betanaphthol	Fast sulfon black pure		Unsatisfactory	Negative entirely	Negative
	(71) Dianisidine + 2 molecules 1.8chlornaphthol3.6 disulfonic acid	145 Diamine brilliant blue G		Unsatisfactory	Lutein cells contain fair numbers of pale orange granules	Unsatisfactory
	(72) Tolidine + 1 molecule Chromotrope acid and 1 molecule 1.8 diamidonaphthylamine 4 monosulfonic acid	154	Pale violet general stain	Corpora lutea reddish	Pale vital granules in lutein cells	Unsatisfactory
	(73) Diamido-azotoluol + 2 molecules Alphanaphthol 4 monosulfonic acid	Red 69936 (Read Holliday)	Pale pink vital stain	Corpora pale pink	Corpora with no granules but germinal epithelium with deep pink granules	Negative

TABLE 36—Concluded.

Table showing vital dyes employed to stain corpora lutea.

Chemical classification of vital dyes employed	Exact chemical constitution of dye	Name or designation of dye	General vital staining properties	Macroscopic vital stain of corpora as seen in fresh tissue	Presence of vital dye granules in the fresh lutein cells	Fixation and preservation of vital dye granules in sections
Other Azo dyes of unknown constitution	(74)	91 Brilliant Benzoecht violet BL		Unsatisfactory	Negative	Negative
	(75)	90 Brilliant Benzoecht violet 2 RL		Unsatisfactory	Negative	Negative
	(76)	Columbia Echtscharlach 4B extra stark		Light salmon general color, apparently diffuse	Negative	Negative
	(77)	85 Congo violet R		Unsatisfactory	Negative	Negative
	(78)	Diamine brilliant scarlet S	Light diffuse general stain	Light diffuse stain of corpora	No red granules in lutein cells	Light diffuse stain of atresia
	(79)	Diamine fast red		Unsatisfactory	Negative in every respect. SECOND SAMPLE: Negative in every respect	
	(80)	Diamine Azo scarlet 4BL	No general vital stain	No macroscopic stain of corpora	Negative	Negative, even atresia negative
	(81)	Diamine Azo scarlet 8B extra	Pale red general stain	Pale red stain of corpora	Scanty, pale red granules in corpus cells. Germinal epithelium loaded with sharp brilliant scarlet granules	Unsatisfactory
	(82)	Diamine fast scarlet GFF	No general stain	Unsatisfactory	Negative	Negative, even for atresia
	(83)	Diamine fast Bordeaux 6BS	Palest pink general stain	Negative	Negative	Negative

TABLE 37.

Succession of œstrous cycles observed in one hundred animals.

Designation of animal	Length of cycle in days
3725	6, 4, M13, 4, 5, 5, 6, M19, 6, 6, M15, 4, 4, 4, 4, 4, 5, 4, 4, 4, 5, M19, 3, 4, 5, 4, 6, 4, 4, 7, 4, 4, 7, 4, 6, 15, 8, 5, 4, 6, 5, 5, 4, 11.
3778	4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 6, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4.
3803	12, 23, 9, 8, 5, 5, 5, 5.
3809	10, 8, 11, 3, 14, 12, 8, 9, 5.
3810	4, 8, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4.
3816	4, 4, 4, 4, 5, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4.
3817	4, 9, 6, 5, 5, 5, 5, 5, 5, 5, 4, 4, 5, 5, 5, 5, 4.
3821	7, 5, 13, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5.
3841	5, 4, 5, 5, 4, 4, 5, 5, 4, 4, 6, 10, 4, 6, 4, 4, 4, 4, 5.
3843	5, 14, 5, 4, 5, 5, 4, 5, 5, 4, 4, 4, 6, 5, 4, 4, 5, 5.
3848	4, 6, 5, 5, 5, 4, 5, 4, 4, 5, 4, 4, 4, 4, 4.
3852	14, 7, 4, 5, 4, 5, 5, 7, 6, 14, 7, 4, 6, 4, 4.
3857	13, 11, 4, 3, 9, 9, 5, 5, 5.
3862	9, 8, 5, 6, 5, 5, 5, 4, 5, 5, 5, 5, 5, 5, 5.
3871	5, 5, 5, 7, 6, 14, 20, 5, 5, 5.
3879	11, 4, 5, 4, 4, 4, 4, 4, 4, 5, 4, 15, 4, 4, 4.
3882	10, 4, 5, 5, 8, 11, 12, 5, 5, 5, 6, 5, 5.
3827	4, 5, 5, 4, 4, 4, 4, 4, 4, 15, 4, 4, 4, 3, 4, 4, 4, 4, 4, 4, 4, 4.
3907	4, 5, 5, 5, 5, 4, 4, 4, 4, 4, 4, 4, 4, 5, 3, 4, 5, 5, 4, 4, 4.
3912	5, 5, 4, 4, 5, 4, 5, 4, 3, 12, 4, 4, 3, 5, 6, 4, 6, 3, 3, 7.
3914	4, 5, 5, 5, 6, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 19.
3919	5, 5, 3, 5, 5, 6, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 5, 3.
3954	5, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4.
3955	5, 6, 15, 5, 6, 6, 5, 4, 5, 6, 4, 19, 4.
3956	5, 6, 5, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 6. 6,
3957	4, 4, 4, 5, 5, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4.
3988	16, 4, 4, 5, 4, 6, 5, 5, 5, 5, 5, 5, 5, 5, 4.
3989	10, 6, 5, 5, 5, 5, 6, 9, 13, 4, 5, 7, 4.
3991	7, 11, 8, 7, 7, 8, 7, 10, 8, 8, 8.
3993	8, 6, 5, 4, 6, 8, 20, 7, 9, 4.
3994	5, 5, 5, 5, 4, 6, 4, 3, 3, 4, 4, 4, 4, 4, 4, 4, 5, 5, 4, 4, 5.
3995	6, 5, 7, 5, 5, 5, 5, 5, 6, 5, 5, 6, 6, 4, 5, 5, 5.
3996	4, 6, 4, 6, 4, 5, 4, 4, 4, 4, 5, 5, 4, 5, 5, 4, 4, 4, 4, 4, 4, 5, 4, 4, 4, 5, 5.
3997	8, 9, 6, 10, 9, 8, 7, 5, 5, 6, 7, 6, 10, 18, 7, 10, 3, 2, 3, 2, 5, 6, 5, 6, 6, 6, 6.
3998	7, 6, 4, 5, 5, 5, 5, 6, 6, 5, 6, 4, 3, 4, 4, 4, 4, 4, 4, 4, 4, 6, 4, 3, 3, 3, 4, 4, 6.
3999	7, 11, 4, 5, 5, 5, 5, 5, 5, 5, 5, 6, 4, 4, 4, 4, 4, 4, 4, 6, 3, 5, 4, 5, 4, 4.
4000	9, 6, 6, 4, 6, 4, 8, 5, 7, 4, 5, 4, 6.
4001	5, 5, 5, 5, 4, 4, 5, 4, 4, 4, 5, 4, 5, 4, 5, 5, 5, 5, 4, 4.
4002	5, 5, 5, 5, 5, 6, 4, 4, 4, 7, 3, 5, 6, 4, 4, 4, 3, 4, 6.
4003	13, 5, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 17, 3, 4.
4004	4, 4, 5, 5, 5, 4, 5, 5, 7, 4, 5, 4, 4, 6, 5, 4, 4, 7.
4005	4, 4, 4, 5, 5, 5, 10, 4, 5, 4, 5, 8, 5, 4, 8, 5.
4006	12, 4, 6, 13, 6, 5, 5, 5, 6, 5, 5, 3, 4, 4, 4, 5, 5, 4, 4, 4, 15, 5, 8, 3, 6, 4, 4, 5, 5, 5, 3, 4.
4007	4, 5, 5, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 5, 4, 4, 4, 4.
4008	8, 6, 5, 4, 6, 4, 5, 6, 5, 5, 5, 5, 5, 5, 5, 5.
4009	5, 5, 4, 5, 6, 6, 6, 6, 6, 4, 8, 4, 5, 5, 5, 5.
4010	10, 3, 3, 6, 6, 4, 4, 6, 5, 7, 7, 4, 6, 7, 8.
4011	3, 6, 13, 9, 5, 5, 5, 5, 5, 5.

TABLE 37—(*Concluded*)

Designation of animal	Length of cycle in days
4012	10, 7, 4, 2, 5, 6, 5, 5, 5, 5.
4015	14, 9, 6, 6, 5, 6.
4021	10, 8, 5, 10, 6, 5, 6, 5.
4024	39, 19, 3, 6, 6, 6, 6, 5, 2, 2.
4025	11, 2, 4, 16, 5, 3, 12, 5, 4, 4, 4, 4, 4, 4, 4, 4, 5, 5, 3.
4027	17, 6, 3, 6, 5, 4, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 14, 4, 4, 4, 4, 5.
4028	4, 4, 10, 9, 4, 8, 6, 5, 4.
4029	13, 3, 11, 4, 9, 5, 4, 6, 4, 6.
4030	12, 5, 8, 5, 8, 5, 6, 7, 8, 5, 4, 5, 5, 7, 4, 5, 3, 6, 4, 5, 6, 4, 4, 4, 4, 4, 5, 5, 5, 6, 6, 5.
4031	3, 8, 17, 11, 8, 5, 4, 5, 6, 4, 4, 4, 4, 4, 5, 6, 4, 6, 4, 4, 7, 5, 5, 4, 4, 4, 4, 6, 3, 5, 4, 5.
4032	5, 6, 4, 6, 6, 6, 4, 4, 6, 4, 5, 5, 4, 5, 5, 6, 4, 5, 5, 5, 5, 5, 4, 5, 4, 4, 6, 4, 5, 6, 5.
4033	5, 5, 4, 5, 4, 4, 4, 4, 5, 4, 4, 4, 4, 4, 5.
4034	10, 19, 6, 4, 4, 5, 4, 5.
4036	4, 5, 5, 10, 6, 4, 5, 5, 6, 4, 5, 6.
4037	5, 7, 5, 5, 5, 8, 5, 6, 4, 6, 4.
4038	5, 5, 6, 11, 5, 5, 4, 6, 4, 5, 5.
4042	9, 12, 9, 9, 7, 4, 6, 5, 4, 4, 4.
4059	15, 15, 16, 5, 7, 5, 7, 5, 5, 4, 5, 8, 4, 4, 5, 6.
4060	7, 14, 17, 5, 5, 5, 5.
4061	11, 9, 7, 8, 6, 7, 4, 6.
4064	7, 5, 9, 3, 12, 5, 5, 4, 6, 5, 4, 4, 4, 4, 4, 4, 4, 3, 5, 4, 4, 4, 4, 4, 4, 4, 5, 4, 4, 4, 5, 4, 5, 4, 4, 3.
4065	11, 28, 9, 6, 4, 5.
4066	9, 5, 6, 5, 5, 14, 6, 5, 5.
4067	9, 4, 5, 7, 5, 4, 6, 6, 4, 6, 7, 4, 5, 4, 5, 4, 6, 4, 4, 3, 6, 4, 4, 4, 5, 6, 6, 4, 6, 7, 7.
4069	5, 5, 7, 12, 14, 5, 5, 5, 5.
4070	10, 6, 6, 22, 7, 4, 5.
4232	6, 3, 4, 4, 4, 4, 4, 4.
4234	5, 4, 5, 4, 6, 4, 4, 4.
4235	5, 5, 5, 4, 6, 4, 5.
4236	4, 4, 4, 4, 4, 4, 4, 4, 4.
4237	6, 7, 4, 7, 5, 5.
4238	4, 5, 5, 5, 4, 5, 5, 5.
4239	5, 5, 4, 5, 6, 5, 5, 5.
4402	17, 5, 6.
4407	10, 9, 5, 6, 10, 6, 5, 6, 5.
4409	6, 11, 9, 5, 7, 7, 6, 5, 4, 4, 4.
4509	11, 6, 7, 6, 5.
4515	8, 14, 6, 7, 6, 6, 5, 5, 6, 5, 4.
4517	5, 17, 4, 9, 5, 5, 5, 5.
4519	9, 4, 8, 5, 5, 5, 5, 6.
4522	8, 4, 5, 6, 7, 5, 5.
4527	14, 10, 3, 5, 6, 4, 5, 4, 6, 5.
4534	15, 29
4537	8, 7.
4538	16, 3, 3, 5, 3.
4540	18, 8, 5, 5, 5, 5, 5, 7, 5, 3.
4541	13, 13, 13.
4550	10, 9, 4, 6, 7, 5, 5.
4562	10, 10, 9, 9, 7.
4567	18, 6.
4569	10, 19.
4578	4, 7, 4, 9, 7, 7.

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